

The influence of food quality (P:C ratio) on RNA:DNA ratio and somatic growth rate of *Daphnia*

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Abstract

Growth experiments with juvenile *Daphnia galeata* were performed to investigate how fast their RNA:DNA ratio responds to changes in food quality, the relationship between RNA:DNA ratio and somatic growth rate, and the effect of food quality (P:C ratio) on the RNA:DNA ratio. RNA and DNA concentrations in individual daphnids were measured with a single-dye (RiboGreen) fluorometric method. Algae were cultured in chemostats and different P:C ratios were obtained by altering the dilution rate and the P content of the medium. The RNA:DNA ratio of the daphnids responded within 5 h to differences in food quality. The RNA:DNA ratio was highly correlated with the somatic growth rate ($r^2 = 0.94$), and the RNA:DNA ratio increased with increasing food P:C ratio below a threshold P:C ratio (by atoms) of approximately 0.005 (C:P \approx 200). The response in RNA:DNA ratio to changes in food quality is rapid and consistent with previously reported patterns for somatic growth rate. This enables short-term experiments that reduces the problems with keeping both food quantity and quality constant during incubation. Analysis of zooplankton RNA:DNA ratios in short-term experiments has a potential for addressing questions about when and where food quantity, quality, or both limit zooplankton growth in nature.

Zooplankton production has often been considered to be energy (carbon)-limited. However, recent empirical and theoretical studies suggest that food quality is an important factor for the regulation of zooplankton production (Sterner and Hessen 1994; Gulati and DeMott 1997; Sterner and Schulz 1998). The basic reason for the decrease in growth rate is that the gross growth efficiency of the consumer decreases if the food contains suboptimal concentrations of essential nutrients. Both elemental imbalances in terms of C:N:P ratios (Sterner and Hessen 1994; Elser et al. 2000a) and low concentrations of essential biochemical compounds, such as some highly unsaturated fatty acids (Brett and Müller-Navarra 1997; Müller-Navarra et al. 2000), have been inferred to cause the low quality of the food. Most research on the relationship between food quality and zooplankton growth so far has been performed in laboratories. More knowledge about the importance of food quality in situ is therefore needed. One obstacle for collecting that type of information is the methodological problems of measuring zooplankton production. A common approach has been to measure the growth in absence of predators, either in situ or in the laboratory (Vijverberg 1989). To measure the change in biomass over time in that type of experiment require long incubations, typically 3–10 d, which makes it difficult and tedious to keep important experimental conditions such as food quantity and food quality constant. The method thus has drawbacks that restrict its suitability for estimating zooplankton production.

An alternative method would be to measure the instantaneous growth rate, and it would preferentially respond rather quickly to changes in feeding conditions. Such snapshot estimates of zooplankton growth can be accomplished either by measuring biosynthetic rates or capacities such as incorporation of chitin (Roff et al. 1994), chitinase activity (Sastri and Roff 2000), or RNA concentration (Båmstedt and Skjoldal 1980). The rationale for using the RNA concentration as a growth rate index is that fast growth requires a high cellular concentration of ribosomal RNA, which constitutes a major fraction of the total RNA (Elser et al. 1996). It has been demonstrated that the RNA content, either expressed as RNA per dry weight or RNA:DNA ratio, is correlated with growth rate in bacteria (Leick 1968; Koch 1970) as well as in eukaryotes such as algae (Dortch et al. 1983), invertebrates (Sutcliffe 1970; Båmstedt and Skjoldal 1980; Saiz et al. 1998), and fish larvae (Buckley 1984; Folkvord et al. 1996). Although the relationship between RNA:DNA ratio and growth rate is both species specific (Dagg and Littlepage 1972), stage specific (Wagner et al. 1998), and temperature dependent (Saiz et al. 1998), it may have a potential for being a useful measure of zooplankton growth both in situ and in short-term experiments. For example, it has been shown the RNA:DNA ratio of marine copepods is correlated with egg production rate and that the RNA content is affected by changes in food quantity (Saiz et al. 1998; Wagner et al. 1998). Whether a reduced growth rate due to low food quality also results in a decrease in the RNA content has, to our knowledge, not been investigated.

In this paper, a single-dye fluorometric method for measuring the RNA and DNA concentrations in individual zooplankters is described. The method is then applied to growth experiments aimed at investigating the relationship between RNA:DNA ratio and somatic growth rate of *Daphnia galeata*, the effect of food quality (P:C ratios) on the RNA:DNA ratio, and the speed at which the RNA:DNA ratio responds to changes in food quality.

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Materials and methods

Cultures and chemical analyses—Stock cultures of *Daphnia galeata* isolated from subalpine Lake Ännsjön were fed *Rhodomonas lacustris* ad libitum for several generations before the experiments. The daphnids were kept in modified 3×L16 medium (Lindström 1991) in 250-ml bottles at 16.5°C. Cohorts of 2-d-old juveniles were used in the experiments. *Rhodomonas* was grown in batch cultures in 5×L16 medium (Lindström 1991) at 16.5°C, 30 $\mu\text{E m}^{-2} \text{s}^{-1}$, and an 18:6 light:dark (LD) photoperiod. The green alga *Scenedesmus quadricauda* was grown in 0.5-L chemostats with Z8' medium (Ahlgren 1977). The algae were grown at 17.5°C, 46 $\mu\text{E m}^{-2} \text{s}^{-1}$, and an 18:6 LD regime. The cultures were stirred with a magnetic stirrer every 5 min and continuously aerated. Different P:C ratios were obtained by changing the dilution rate (range: 0.18–0.64 d^{-1}) and the P content of the medium (10, 50, or 100% of original P concentration; KCl was added to keep potassium concentration and ion strength constant). Known volumes of algal cultures were filtered on precombusted (3 h at 530°C) glass fiber filters (MGC, Munktell) for analysis of the C and P content. Carbon was analyzed with a LECO CHNS-932 analyzer, and P was analyzed by the molybdate blue reduction (Murphy and Riley 1962) after hot acidic oxidative hydrolysis with 5% $\text{K}_2\text{S}_2\text{O}_8$. For routine dilution of *Scenedesmus* cultures, a relationship between algal C concentration and in vivo optical density ($\lambda = 680 \text{ nm}$, 1-cm glass cuvette) was established.

Nucleic acid quantification—RNA and DNA were extracted and analyzed using the fluorochrome RiboGreen in combination with RNase treatment (Fig. 1). RiboGreen is a sensitive cyanine dye that specifically binds to RNA and DNA (Jones et al. 1998). RiboGreen, lambda DNA, ribosomal RNA (16S and 23S from *E. coli*) and 20×TE buffer (200 mM Tris-HCl, 20 mM EDTA, pH 7.5, RNase- and DNase-free) were supplied by Molecular Probes. Ribonuclease A from bovine pancreas (RNase), Triton X-100, protease (type XXVII, 8 units mg^{-1} , RNase- and DNase-free) and diethyl pyrocarbonate were supplied by Sigma. Nuclease-free water was prepared by adding diethyl pyrocarbonate (0.1% final concentration) to deionized water and by incubating in a water bath at 37°C for 2 h followed by autoclaving at 120°C for 20 min. 1×TE buffer was prepared by diluting the 20×TE buffer with nuclease-free water. RiboGreen reagent solution was prepared by diluting the stock solution 200-fold with 1×TE buffer. The reagent solution was kept dark and cold and was used within a few hours.

RNA and DNA were extracted from individual zooplankters in 1×TE buffer containing Triton X-100 (0.1% final concentration) and protease (0.1 mg ml^{-1} final concentration). Buffer (50 μl) was added to each eppendorf vial containing one zooplankton individual. The animal was thoroughly crushed with a plastic pellet pestle (Kontes). The head of the pestle was cut off with a pair of nippers and left in the vial. Another 220–450 μl of the buffer was added. The volume added depended on the size of the animals. Different extraction volumes were

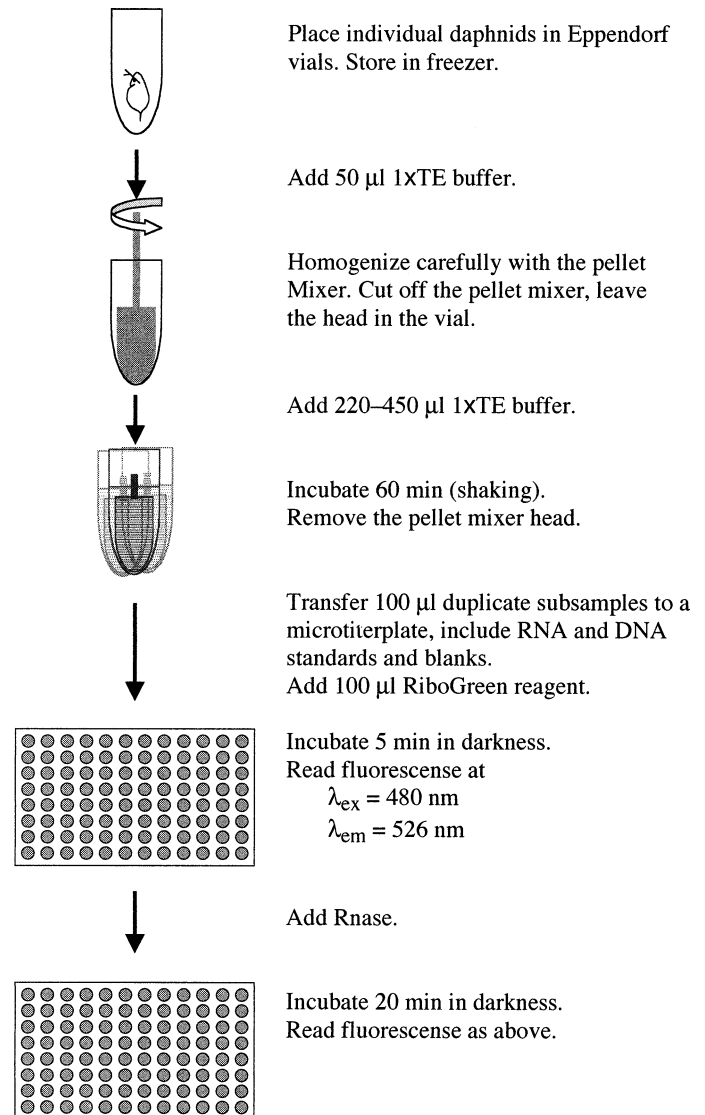


Fig. 1. Outline of the nucleic acid extraction and analysis. See text for details.

used to keep RNA and DNA concentrations within the linear region of the analysis but still substantially above the detection limit. After the addition of the buffer, the samples were thoroughly shaken for 1 h at room temperature. After removing the head of the pellet pestle, duplicate 100- μl subsamples and standards (containing mixtures of 0–600 ng DNA ml^{-1} and 0–800 ng RNA ml^{-1} in 1×TE buffer with protease and Triton X-100) were transferred to a black 96-well microplate (MaxiSorp FluoroNunc, Nalgenunc International). RiboGreen reagent solution (100 μl) was added, and the samples were gently mixed for 5 min in darkness. Fluorescence was then measured in a spectrofluorometer with microplate reader (FluoroMax-2 with MicroMax, Instruments S.A.) at 480 nm excitation wavelength (band width 4 nm), 526 nm emission wavelength (band width 5 nm), and an integration time of 1 s. After the fluorescence measurements, 25 μl RNase solution (4 Kunitz U ml^{-1}) was added to all samples and standards. The

microtiterplate was gently shaken for 20 min in darkness, and the fluorescence was subsequently measured as above.

Experimental setup—The animals were kept in phosphorus-free medium in 100-ml glass bottles with five to seven animals in each bottle. The algal concentration was ≥ 2 mg C L⁻¹ (i.e., well above the incipient limiting level). The animals were moved to fresh medium every 24 h using a 5-ml pipette. To keep the algae from aggregating on the bottom, the bottles were fastened to an axis rotating at 1 rpm for 15 min every hour. The bottles were completely filled with medium to decrease turbulence and the risk of daphnids getting caught in the surface tension. Neither the rotation nor the lack of atmospheric contact seemed to affect the daphnids negatively. Daphnids enclosed in 250-ml bottles in the same way, given enough food, were able to survive and reproduce for more than 10 d without the bottle being opened (Sundbom and Vrede 1997). The experimental temperature was 14.2–14.5°C. At the beginning of each experiment, individual daphnids were randomly sampled and their length (total body length excluding the caudal setae) and RNA and DNA content were measured. In some experiments, additional individuals were sampled for determination of initial C biomass. How fast the RNA:DNA ratio changes in response to changes in food quality was investigated in two time-course experiment in which the RNA:DNA ratio of the daphnids was measured after 5 h (one experiment only), 12 h, and 24 h. The animals were fed either *Rhodomonas* (P:C = 0.0037 [by atoms]) or *Scenedesmus* (P:C = 0.0020, 0.0021, or 0.016 [by atoms]). For each treatment and time, the RNA and DNA content was analyzed in 8–15 individual daphnids. The relationships between food quality (P:C ratio), specific growth rate, and RNA:DNA ratio was investigated in a series of experiments where daphnids were fed algae with different P:C ratios. These experiments lasted for 3 d. In addition, data from 24 h in the time-course experiments were also used for the analysis of the relationship between P:C ratio and RNA:DNA ratio. According to stoichiometric mass balance models (e.g., Brett et al. 2000, and references therein), we expect a transition from P- to C-limitation of *Daphnia* growth at a threshold elemental ratio (TER). At food P:C ratios below the TER, the animals are supposedly P-limited, whereas they become C-limited at higher P:C ratios. Maximum growth rate should, according to these models, occur at all food P:C ratios higher than the TER.

Calculations and statistical analyses—RNA and DNA standard curves were calculated by fitting multiple linear regressions to the fluorescence data from the standards both before and after RNase treatment.

$$\text{fluorescence} = a + b[\text{RNA}] + c[\text{DNA}]$$

The DNA concentration was calculated from fluorescence measurements after RNase treatment (i.e., when $b = 0$). The contribution of DNA to the total fluorescence before RNase treatment was then subtracted and the RNA concentration was subsequently calculated. The specific growth rate (r) of *Daphnia* was calculated assuming exponential growth.

$$r = (\ln C_t - \ln C_0) t^{-1}$$

C_t and C_0 are carbon content at times t and 0, respectively, and t is the duration of the experiment. Standard curves, nucleic acid concentrations, growth rates, descriptive statistics, one-way analyses of variances (ANOVAs) and t -tests were calculated with Microsoft Excel 98 for Macintosh. Piecewise linear regression, linear regression, and factorial ANOVA were calculated with JMP 4.0 for Macintosh. The piecewise linear regression was implemented in the nonlinear fit platform of JMP using the Gauss–Newton method with step halving. The parameters a , b , and c were estimated using the following model.

$$\begin{array}{ll} \text{if } P:C < c & Y = a + b \cdot P:C \\ \text{else} & Y = a + b \cdot c \end{array}$$

Y is either the RNA:DNA ratio or specific growth rate of *Daphnia*, $P:C$ is the algal P:C ratio, a is the intercept, b is the slope of the left part of the curve, and c is the TER, above which the RNA:DNA or specific growth rate is constant. In cases where there were alternative stable solutions, the results reported here are those with the lowest residual sum of squares.

Results

Before the RNase treatment, the fluorescence increased linearly both with RNA and DNA concentration within the concentration range used (Fig. 2A,B). RNase treatment resulted in an almost complete loss of RNA fluorescence, but no effect on the DNA fluorescence (Fig. 2C,D). Analysis of residuals from several measurements of standards revealed no systematic variation either with DNA or RNA concentration. The coefficient of variation between duplicate samples (after subtraction of the blind) was on average 2.0 and 1.2% for standards and 1.3 and 5.4% for *Daphnia* samples, before and after RNase treatment, respectively.

In the time-course experiments, feeding the daphnids with food of different quality resulted in a significant difference in the RNA:DNA ratio, with higher ratios in the treatments with higher P:C (Fig. 3, Table 1). There was a temporal variation in the RNA:DNA ratio, but no interaction between treatment and time. Thus, the difference in RNA:DNA ratio developed rapidly (≤ 5 h) and remained throughout the experiments. For some reason that is unclear, the RNA:DNA ratios were substantially lower after 12 h in experiment A than in experiment B, even though the daphnids were fed with *Scenedesmus* of similar P:C.

In the growth vs. food quality experiment, the similarity in the initial conditions of the experimental runs was tested for *Daphnia* carbon biomass, total body length, and RNA:DNA ratio (Table 2). None of the parameters differed significantly between the experimental runs ($P_{\text{carbon}} = 0.31$, $P_{\text{length}} = 0.67$, and $P_{\text{RNA:DNA}} = 0.82$; one-way ANOVA). After 3 d incubation, both C content and RNA:DNA ratio varied substantially between treatments: 5.6–7.9 $\mu\text{g C individual}^{-1}$ and 9.3–14.6 $\mu\text{g RNA } (\mu\text{g DNA})^{-1}$, respectively (Table 3). There was a strong linear relationship between somatic growth rate and RNA:DNA ratio ($R^2 = 0.94$, $P = 0.001$)

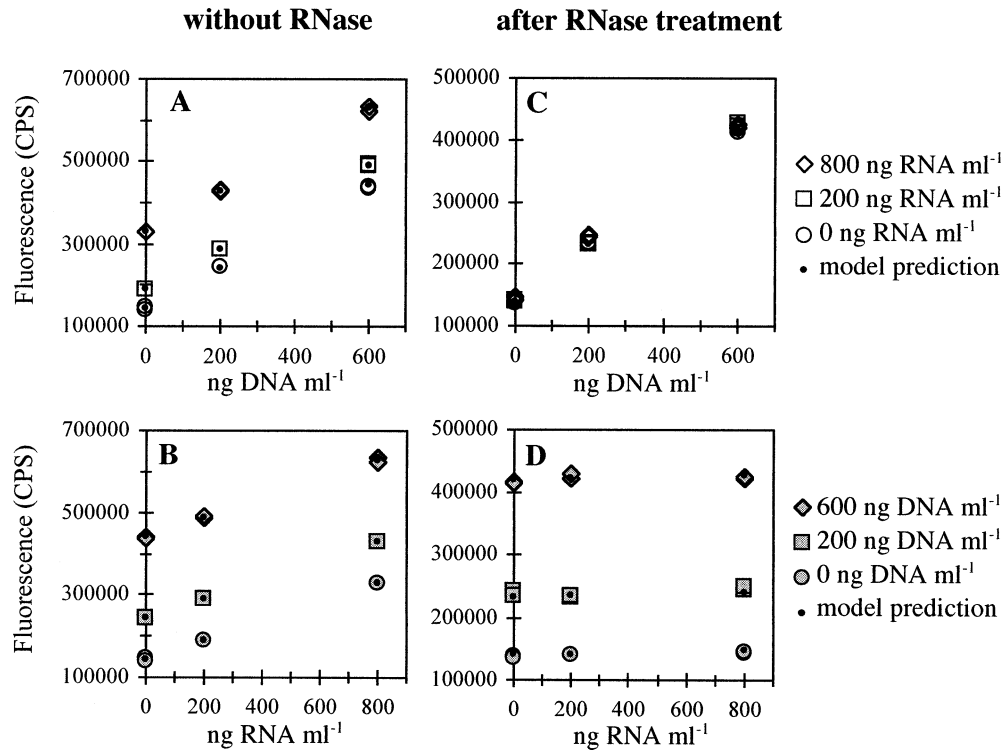


Fig. 2. Typical results from fluorescence measurements of RNA and DNA standards with RiboGreen. (A, B) before RNase treatment; (C, D) after RNase treatment. Each combination of RNA and DNA was measured in duplicate. Predicted values were calculated from slopes and intercepts estimated by multiple linear regressions. Fluorescence measurements are given as counts per second (CPS).

(Fig. 4). Extrapolation of the regression line to zero growth rate gives an RNA:DNA ratio of 6.2.

The RNA:DNA ratio increased with increasing food P:C ratio, and the best fit of the piecewise linear regression to the data from the 3-d incubations indicates that TER is at an algal P:C ratio of 0.0047 ± 0.0007 (estimate \pm approximate SE, by atoms), corresponding to a C:P of 212:1 (Fig. 5A). Above the TER, the RNA:DNA ratio remained stable at approximately $14 \mu\text{g RNA } (\mu\text{g DNA})^{-1}$ (Fig. 5A). When results from 24 h in the time-course experiment were included in the data set, the TER was 0.0055 ± 0.008 (C:P = 180:1), and the maximum RNA:DNA ratio was $14 \mu\text{g RNA } (\mu\text{g DNA})^{-1}$ (Fig. 5A). A similar pattern was found for the specific growth rate, which was positively related to algal P:C ratio below a TER of 0.0045 ± 0.0003 (C:P = 220) and remained stable at approximately 0.24 d^{-1} above the TER (Fig. 5B).

Discussion

RNA and DNA quantification—The RiboGreen method described in this paper produces reproducible results with a sensitivity and precision that enables the analysis of nucleic acids in individual zooplankton. Jones et al. (1998) reported a linear increase in fluorescence for nucleic acid concentrations up to $1 \mu\text{g RNA ml}^{-1}$ or $0.5 \mu\text{g DNA ml}^{-1}$ when using the high-range (750 nM) RiboGreen assay. However, RNA and DNA were not simultaneously present in that study, and one might expect that the linear range is smaller when both

RNA and DNA are present simultaneously. The analysis of residuals from the multiple linear regressions of fluorescence of standard samples indicate that the linear range is not exceeded when DNA and RNA are simultaneously present at concentrations of $600 \text{ ng DNA ml}^{-1}$ and $800 \text{ ng RNA ml}^{-1}$. In a review of techniques for measuring RNA and DNA in fish larvae, Buckley et al. (1999) pointed out the difficulties in comparing results obtained by different analytical protocols; both the choice of standards as well as the extraction procedure can influence the results. The former problem might not be of large quantitative importance if RiboGreen is used because it has been shown to produce similar results regardless of the type of RNA (Jones et al. 1998). Furthermore, using ribosomal RNA (rRNA) as the standard could reduce this problem. The choice of rRNA is justified by the observation that the major fraction of RNA is ribosomal (Alberts et al. 1983). There is little known about the influence of the type of DNA standard on the result when analyzing DNA with RiboGreen, but in order to make results from different studies comparable, this aspect should not be neglected in future studies. To make the extraction procedure efficient, we added both the tenside Triton X-100 to disrupt the cell membranes and protease to digest DNA- and RNA-binding proteins (both histones and nonhistones), which may reduce the efficiency of the extraction or block the fluorochrome binding sites. The physical disintegration of the animals also turned out to be important. The variation in RNA and DNA content among replicate daphnids was substan-

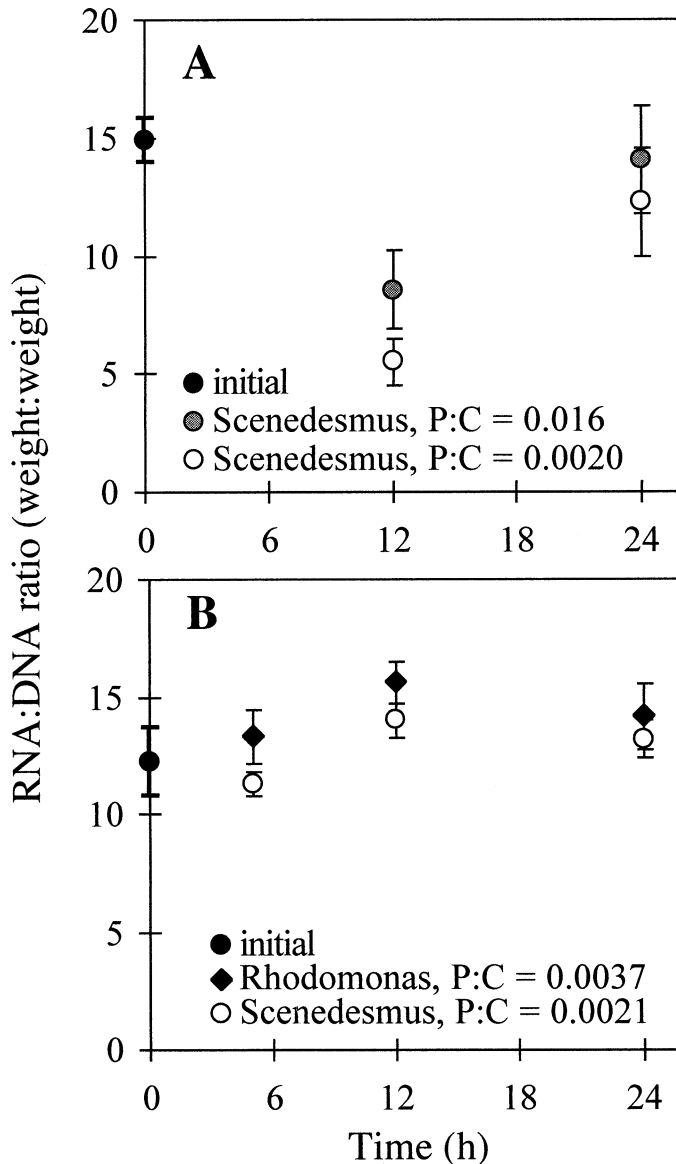


Fig. 3. RNA:DNA ratios of *Daphnia galeata* in the time-course experiments. Algal P:C ratios are by atoms.

tially higher if whole animals were extracted than if the animals were crushed with a pestle. We also tried to disintegrate the animals with an ultrasonicator, but it resulted in a rich production of lather (because of the presence of tenside), which made quantitative pipetting of the sample impossible.

RNA:DNA ratio, growth rate, and food P:C ratio—The somatic growth rate of the daphnids was highly correlated with their RNA:DNA ratio (Fig. 4). This result is well in line with the often-observed positive relationship between RNA content and growth rate (reviewed in the introduction). Both the growth rate and the RNA:DNA ratio increased with increasing algal P:C ratio but remained stable over a threshold value (Fig. 5). Similar relationships between somatic growth rate and food P:C have previously been observed (Sterner 1993; Sundbom and Vrede 1997; DeMott et

Table 1. ANOVA table for the time-course experiments (initial values excluded from the analysis). The response variable is *Daphnia* RNA:DNA ratio. The food suspensions consist of *Scenedesmus* with P:C = 0.0020 (by atoms) or 0.016 (Sce/Sce experiment) or *Scenedesmus* with P:C = 0.0021 or *Rhodomonas* (Rho) with P:C = 0.0037 (Sce/Rho experiment).

Experiment	Source of variation	df	F	P
Sce/Sce	P:C	1	40.1	<0.0001
	Time	1	6.3	0.02
	P:C × time	1	0.4	0.53
	Error	30		
Sce/Rho	P:C	1	8.8	0.0044
	Time	2	9.4	0.0003
	P:C × time	2	0.2	0.81
	Error	56		

al. 1998), but to the best of our knowledge, this is the first observation of a response in RNA:DNA ratio as a result of changing food quality. The estimates of the threshold P:C ratio in this study, ranging from 0.0045 to 0.0055, which correspond to C:P ratios from 180 to 220, are similar to the empirical results of Sterner (1993), but somewhat lower than 250, which is the lower range of the TER of *Daphnia* spp. as calculated by Brett et al. (2000). Although the correlation between algal P content and zooplankton growth rate in this type of experiment does not prove that the daphnids are P limited, because P content can be correlated with other food-quality parameters such as fatty acid content or digestibility, other studies have shown that *Daphnia* growth can be limited by mineral P (Urabe et al. 1997; DeMott et al. 1998; Boersma 2000). The results of the present study, as well as previous studies (e.g., Saiz et al. 1998; Wagner et al. 1998), suggest that RNA:DNA ratios can be useful indicators of crustacean zooplankton growth. However, a number of questions can be raised, pointing out potential pitfalls and directions of future research, which will be addressed herein.

Is the RNA:DNA a better indicator of growth rate than RNA per dry weight (RNA:DW)? The results of Dagg and Littlepage (1972) indicate that even though there is a posi-

Table 2. *Scenedesmus* P:C ratios and initial *Daphnia* carbon content, total body length (excluding the caudal spine), and RNA:DNA ratio in the 72-h experiments (average ± 95% confidence interval). The number within parentheses refers to the number of replicates. Each replicate consists of five individuals (Carbon) or one individual (Length and RNA:DNA).

Algal P:C (by atoms)	Algal C:P (by atoms)	Carbon (μg C individual ⁻¹)	Length (mm)	RNA:DNA (wt:wt)
0.0026	388	3.90±0.44(3)	1.09±0.02(21)	9.7±1.5(6)
0.0032	312	3.44±0.36(3)	1.08±0.02(21)	9.7±1.0(6)
0.0038	265	3.76±0.34(3)	1.08±0.01(21)	9.3±1.3(6)
0.0064	157	3.80±0.80(3)	1.09±0.02(21)	10.7±1.0(6)
0.0139	72	3.98±0.38(3)	1.07±0.03(21)	9.1±0.8(6)
0.0256	39	4.30±0.36(3)	1.07±0.02(21)	10.1±1.9(6)

Table 3. *Scenedesmus* P:C ratios and final *Daphnia* carbon content and RNA:DNA ratio in the 72-h experiments (average \pm 95% confidence interval). The number within brackets refers to the number of replicates, and each replicate consists of five individuals (Carbon) or one individual (RNA:DNA).

Algal P:C (by atoms)	Algal C:P (by atoms)	Carbon ($\mu\text{g C individual}^{-1}$)	RNA:DNA (wt:wt)
0.0026	388	$7.90 \pm 0.84(5)$	$14.6 \pm 0.9(25)$
0.0032	312	$7.24 \pm 1.6(5)$	$13.8 \pm 0.7(25)$
0.0038	265	$7.82 \pm 0.30(5)$	$13.8 \pm 0.7(24)$
0.0064	157	$6.48 \pm 0.62(5)$	$11.7 \pm 0.7(25)$
0.0139	72	$6.44 \pm 1.2(5)$	$11.8 \pm 0.9(25)$
0.0256	39	$5.64 \pm 0.58(5)$	$9.3 \pm 1.3(13)$

tive relationship between somatic growth rate and RNA:DW of *Artemia salina*, there is also a large variation in growth rate at any given RNA:DW ratio. They explained this variation in two ways: the amount of RNA reflects potential, rather than actual growth rate, or periodic changes in, for example, copepod lipid content affects the RNA:DW ratio of the copepod. Extending the reasoning of the first explanation, the amount of rRNA would set a limit on the potential growth rate, but this growth rate cannot be achieved if it is constrained by some nutrient deficiency in the food. This explanation is not plausible in the light of the results from the present study because the RNA:DNA ratio of *Daphnia galeata* reacted quickly to changes in food quality, indicating a rapid change in the amount of rRNA. This rapid response in RNA content is consistent with the observation that protein synthesis is mainly regulated by the number of ribosomes rather than the efficiency of the ribosomes (Nomura et al. 1984). A rapid response (24 h) of the RNA:DNA ratio to changes in food quantity has also been observed for *Acartia grani* (Saiz et al. 1998). The feeding conditions were constant for 3 d in all treatments, and the animals were there-

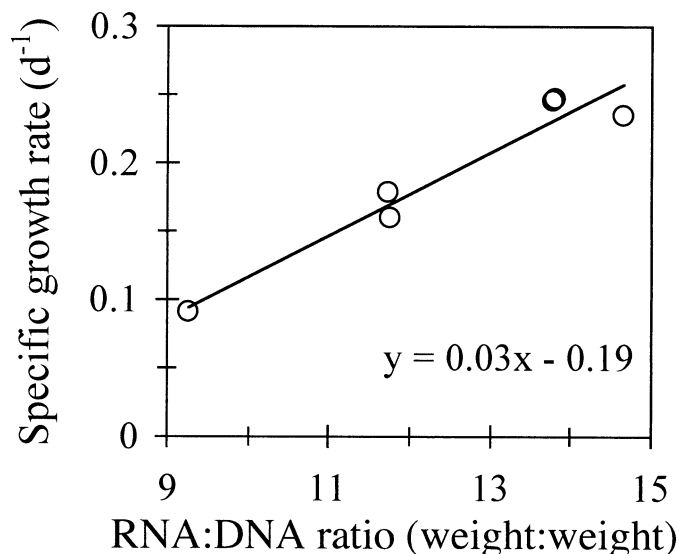


Fig. 4. Relationship between somatic specific growth rate and RNA:DNA ratio of *Daphnia galeata*.

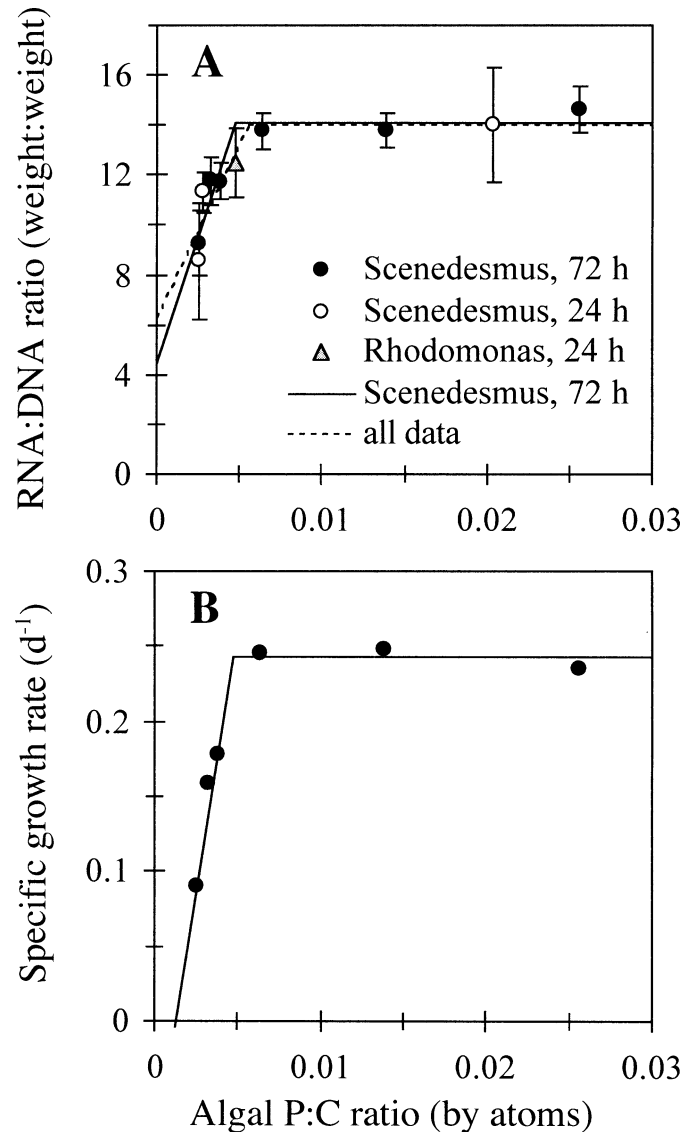


Fig. 5. Relationships between algal P:C ratio and (A) RNA:DNA ratio and (B) specific growth rate of *Daphnia galeata*. The regression lines were fitted using piecewise linear regressions. Error bars show 95% confidence intervals.

fore expected to have time enough to adjust their rRNA content. The second explanation, changes in lipid concentrations, would obviously affect the RNA:DW ratio but not the RNA:DNA ratio. Thus, organic matter that is consumed, but cannot be used for growth because of P deficiency in the food, can be stored for later use. Consequently, the ability of crustaceans to store lipids (e.g., Tessier et al. 1983) can result in a significant variation in the RNA:DW-growth rate relationship. On the other hand, the use of RNA:DNA ratios as a growth rate index has been criticized for not taking into account that either RNA or DNA changes depending on the type of growth, the RNA content being connected with protein synthesis rate, and thus cellular growth, and the DNA content being connected with cellular multiplication (Lucas 1996). Rapid growth with simultaneous cell division and protein synthesis thus would not necessarily be more likely

to result in a higher RNA:DNA ratio than if both cell division and protein synthesis rates were low. Both RNA:DW and RNA:DNA can vary because of factors other than growth rate, and it is at present premature to draw any general conclusions about whether one or the other should be preferred as a growth rate index.

Is the RNA:DNA ratio a general indicator of growth? Based on the arguments that growth rate is intimately connected to protein synthesis rate and that most RNA is rRNA, a change in growth rate should be associated with a change in RNA (Elser et al. 1996). The RNA:DNA ratio is therefore expected to change as feeding conditions change, both in terms of quantity and quality. However, the empirical support for this theoretical conclusion is unfortunately limited. Several studies indicate that food quantity affects the RNA:DNA ratio and the growth rate (e.g., Saiz et al. 1998; Wagner et al. 1998), whereas no studies have addressed the question of whether food quality in general affects the RNA:DNA ratio. Another source of variation in the relationship between RNA:DNA ratio and growth rate is the protein synthesis rate, which like other enzymatic processes is temperature dependent. Empirical data show that the growth rate increases with temperature both in fish (Buckley 1984) and copepods (Wagner et al. 1998). The life cycle of crustaceans, which involves complex events such as molting and even metamorphosis, might also have consequences for the relative rates of cellular multiplication and cellular growth both within and between molts, which in turn affects the RNA:DNA ratio. For instance, it can be speculated that variation in RNA:DNA ratio within the molt cycle might be the explanation for the unusually low RNA:DNA ratios at 12 h in one of the short-term growth experiments (Fig. 3). Furthermore, the RNA:DNA ratio differed substantially between life stages of *Calanus finmarchicus* grown at the same feeding conditions (Wagner et al. 1998). Thus, more research is clearly needed regarding the effect of inter- and intramolt variation in RNA:DNA ratios before the results of the present study can be applied to natural populations of animals of different age. However, in an experimental situation in which all the animals are of the same age, the general picture that emerges is that the RNA:DNA ratio is a good predictor of growth rate. The relationship found in this study between RNA:DNA ratio and growth rate cannot be uncritically applied without further calibration, which should take into account the effects of species, life stage, molt cycle, and temperature. In addition, the generality of the RNA:DNA ratio as an indicator of animal growth should be further considered.

Given the many potential problems with the RNA:DNA method, why should we use it instead of somatic growth rate measurements? First, if properly calibrated to species, stage, and temperature, it can provide a snap-shot index of zooplankton growth rate under natural conditions, which makes tedious in situ incubations unnecessary. The growth rate observed in such experimental incubations can be biased by experimental artifacts, which are avoided using the RNA:DNA method. Second, the rapid response (i.e., from a few hours to a day rather than several days, which is required for measuring biomass increments) in the RNA:DNA ratio to changes in either food quality or food quantity signifi-

cantly reduces the problems associated with keeping both food quantity and quality constant during an experiment or in situ incubation. Third, the relationships between the biochemical composition of organisms and their C:N:P stoichiometry and growth rate are now developing as an important theme in ecological stoichiometry (Elser et al. 1996; Elser et al. 2000b). RNA is important in this context because it has a central role in biosynthesis and thus organism growth, and it has a characteristic C:N:P stoichiometry with a high N content and a very high P content. RNA concentrations in animals are therefore particularly interesting with regard to the question of mineral nutrient limitation of animal growth. Despite the existence of several experimental and correlative studies, knowledge is still relatively poor about when and where different nutrients limit zooplankton growth in nature (Gulati and DeMott 1997; Sterner and Schulz 1998). In addition, only little is known about the effects of short-term variation in food quality (Sterner and Schwalbach 2001). If the results of this study are applicable to other life stages, other species, or both and are corrected for temperature effects, the measurement of RNA:DNA ratios of zooplankton has a potential for answering such questions.

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