SHORT COMMUNICATION

Does female RNA content reflect viable egg production in copepods? A test with the Baltic copepod *Acartia tonsa*

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Biomarkers are very useful for *in situ* assessments of zooplankton growth. In particular, RNA-based methods have been developed to estimate egg production in copepods. However, RNA–growth relationships can potentially depend on a variety of factors, such as egg quality. This study shows that in *Acartia tonsa*, female RNA content reflects egg production irrespective of egg viability, implying that this growth proxy is not applicable for recruitment studies if the proportion of viable eggs fluctuates widely.

KEYWORDS: RNA; egg viability; biomarker; copepods; nucleic acids; egg production; *Acartia; tonsa*

Biomarkers, especially RNA-based indices, have become a useful tool in zooplankton growth assessments, as cellular RNA content is closely related to protein synthesis. Ribosomes, the “protein factories” of the cell, consist of about 50–60% ribosomal RNA (rRNA), which represents about 80–90% of an individual’s RNA. This implies that a higher ribosome number would translate into higher protein synthesis and thus higher growth rates (Elser *et al.*, 2000). However, elevated protein synthesis is not always translated into growth, and different factors may influence the relationship between RNA content and growth (Saiz *et al.*, 1998; Holmborn and Gorokhova, 2008). Measuring egg production rate (EPR) in copepods is the most common to assess growth rate (First *et al.*, 2003) and individual RNA content correlates positively with egg production in copepods, including *Acartia* species (Saiz *et al.*, 1998; Gorokhova, 2003; Holmborn and Gorokhova, 2008). However, as egg viability *in situ* varies, the actual population recruitment would differ from the estimated egg production. Therefore, it is unclear if and how female RNA content is related to egg viability. If the individual 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 egg viability. However, in the cyclopoid copepod *Acanthocyclops vernalis* during oogenesis, when demand for rRNA is high, oocytes supply all the rRNA needed by the embryo (Standiford, 1988). If an inadequate supply of maternal rRNA would compromise egg development, then female RNA content may reflect offspring viability.
To test if egg viability affects the relationship between RNA and EPR in copepods, we analysed individual RNA content (µg ind⁻¹), EPR (eggs female⁻¹ day⁻¹) and percentage of viable eggs (%Viable) in female Acartia tonsa Dana, a free-spawning calanoid copepod. This was done by monitoring these variables in A. tonsa sampled before, during and after a cyanobacterial bloom largely composed of Aphanoizomenon flos-aquae (on an average 61% of the total diazotrophs biovolume) and Nodularia spumigena (≏16%). As negative effects of these cyanobacteria on copepod reproduction (Koski et al., 1999; Kozlowsky-Suzuki et al., 2003) have been observed, we expected both egg production and viability to vary, thus providing an opportunity to examine the effects of egg viability on the relationship between individual RNA content and EPR.

The study was conducted 4 July to 26 September 2007 in the vicinity of the Askö Laboratory, in the northern Baltic proper (58°49′ N, 17°39′ E). Phytoplankton samples were collected bi-weekly from 0 to 20 m and treated according to the methods in HELCOM monitoring guidelines (Helcom, 2008). Copepods were collected from the upper 10 m by vertical hauls using a WP-2 net (mesh size 200 µm, diameter 58 cm). Surface water temperature (Temp; 15.5–20.5°C) was measured on each sampling occasion. Copepods were brought to the laboratory in insulated containers (20 L), where adult females were sorted using a wide-mouth pipette, placed individually in 12-well microplates (Corning Costar, Corning NY, USA) filled with 5 mL of 6-indoacetamido-2-pentylcarbazole (Molecular Probes, Inc., Eugene, OR, USA), high-range assay (Gorokhova and Kyle, 2002) and a fluorometer; FLUOstar Optima (BMG Labtechnologies). Standard RNA concentrations ranged from 0.08 to 1.25 µg RNA mL⁻¹. Prior to RNA analysis, the female prosome length (PL; 0.78 mm ± 0.06, mean ± SD, n = 65, range = 0.61–0.91 mm) was measured using an inverted microscope (×80; Wild 40, Heerbrugg) equipped with an ocular micrometer.

Individual RNA content was measured using the nucleic acid fluorescent dye RiboGreen™ (Molecular Probes, Inc., Eugene, OR, USA), high-range assay (Gorokhova and Kyle, 2002) and a fluorometer; FLUOstar Optima (BMG Labtechnologies). Standard RNA concentrations ranged from 0.08 to 1.25 µg RNA mL⁻¹. Prior to RNA analysis, the female prosome length (PL; 0.78 mm ± 0.06, mean ± SD, n = 65, range = 0.61–0.91 mm) was measured using an inverted microscope (×80; Wild 40, Heerbrugg) equipped with an ocular micrometer.

To examine effects of egg viability on the relationship between the female RNA content and EPR, a forward stepwise multiple regression analysis was applied, using STATISTICA 8.0 (StatSoft, Inc., 2007), with individual RNA content as a dependent variable and %Viable, EPR, PL and Temp as independent variables. Visual inspection of distribution plots, Kolmogorov–Smirnov test of normality and quantile plots showed normal distribution of the residuals or deviations from normal distribution of the response variable and the residuals. To test the validity of our assumption that cyanobacteria negatively affect copepod reproduction, Spearman rank–correlation coefficients were calculated between total biovolume of filamentous cyanobacteria at the time of sampling and reproduction variables (EPR and %Viable). Data are reported as mean ± SD, significance was accepted for P < 0.05.

Experimental mortality among females was low (≏5%) and over 70% of them produced eggs during the 24 h incubation. Whereas EPR was relatively low (5.4 ± 4.0 eggs female⁻¹ day⁻¹), with a clear decrease in August, egg viability was generally high (86 ± 24%), with no apparent trend during the study period (Fig.1). Our values on egg viability (measured as %Viable) correspond well with egg viability measured as hatching success of Acartia tonsa in other studies (Holste and Peck, 2006). The observed variation in egg viability was not related to the abundance of filamentous cyanobacteria, as indicated by the lack of correlation between cyanobacteria and %Viable (P > 0.05). In contrast, filamentous cyanobacteria were significantly and negatively correlated with EPR, albeit the correlation was weak (P < 0.05; R = -0.27). The RNA content in females was 0.17 ± 0.07 µg ind⁻¹ (n = 65), which is comparable with other Acartia species (Gorokhova, 2003; Holmborn and Gorokhova, 2008), with a concave-up trend during the study period.

The only significant predictors for RNA were PL and EPR, whereas effects of both %Viable and Temp were not significant (Table 1). This implies that in Acartia tonsa feeding on summer plankton assemblages, egg viability status does not influence the relationship between female
RNA content and EPR. This may indicate that the amount of RNA required to produce an egg is relatively constant, irrespective of its viability status. Also, in line with earlier experimental studies, we found that the presence of cyanobacteria negatively affected copepod EPR, (e.g. Koski et al., 1999; Kozlowsky-Suzuki et al., 2003), with no evidence for a detrimental impact on egg viability.

A possible reason for female rRNA to reflect egg production but not egg viability could be related to the importance of compounds other than RNA and proteins for egg viability, such as polyunsaturated fatty acids (Shin et al., 2003), sterols (Crockett and Hassett, 2005) and phytoplankton toxins and exudates (e.g. Frangopulos et al., 2000). For example, if egg viability is affected by deficiency of lipid allocation to egg yolk, rRNA content of a female is unlikely to indicate egg viability, because rRNA is mostly related to protein metabolism rather than lipid production/allocation.

Another explanation, for the lack of an effect of egg viability on the relationship between RNA and EPR, is suboptimal growth conditions for *Acartia tonsa* during the study period. The EPR values observed were relatively low for this species worldwide but similar to those observed previously in the Southern Baltic Sea (5.8 ± 4.0 eggs female⁻¹ day⁻¹ in September; and 8.5 ± 3.3 in June–July; Schmidt et al., 1998). Moreover, the significant negative correlation between EPR and cyanobacteria indicates that the relatively low EPR may be related to suboptimal feeding conditions *in situ*, where filamentous cyanobacteria contributed ~20% to the total autotroph biovolume. These cyanobacteria have been found to negatively affect zooplankton feeding and EPR, both as a result of direct grazing and decreasing palatability of other prey by protease inhibitors (Schwarzenberger et al., 2010). Also, low food availability during the experiment may have resulted in lower EPR (Parrish and Wilson, 1978) and/or RNA, but the extent of these effects is unknown. Finally, temperature and salinity during our study (16.7 ± 2.5°C and 6–7 in Practical Salinity Units) could have been suboptimal for this species (Holste and Peck, 2006). In stressed animals, RNA demand might increase to counteract the stress by elevated maintenance costs and/or stress protein production. In this case, the proportion of RNA that supports egg production would decrease, while the differences in RNA allocation to non-viable versus viable eggs would not be detected. To test if this is the case, analysis of relationships between RNA content in eggs and nauplii and their viability status would be most useful. Also, controlled laboratory experiments are necessary to test if and how abiotic stressors that have a potential to influence the proportion of viable eggs in copepods (e.g. temperature, salinity: Holste and Peck, 2006; hypoxia: Lutz et al., 1994 and environmental contaminants: Gorokhova, 2010) may affect the relationship between RNA and EPR. In these tests, a possible influence of stress on RNA–growth regressions should be assessed in concert with gross growth efficiency.

It is necessary to separate factors affecting egg viability via female body condition from those directly affecting eggs when released. Suboptimal salinity, temperature, UV-light, hypoxia, toxins and contaminants may all affect egg development without affecting the female and are therefore not reflected in female RNA content. Also fertilization success, while affecting egg

### Table I: *Acartia tonsa*. Results from multiple linear regression analysis

<table>
<thead>
<tr>
<th>Variable</th>
<th>Beta ± SE</th>
<th>P level</th>
<th>R²</th>
<th>Adjust R²</th>
<th>SE estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNA = −0.323 + 0.597 PL + 0.004 EPR</td>
<td></td>
<td>0.0000</td>
<td>0.39</td>
<td>0.37</td>
<td>0.05</td>
</tr>
<tr>
<td>Intercept</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.0008</td>
</tr>
<tr>
<td>PL</td>
<td>0.51 ± 0.10</td>
<td></td>
<td>0.0000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPR</td>
<td>0.26 ± 0.10</td>
<td></td>
<td>0.0149</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Acartia tonsa*. RNA, individual RNA content (µg RNA female⁻¹); PL, prosome length (mm) and EPR, egg production rate (eggs female⁻¹ day⁻¹). Beta ± SE, beta weights with standard errors; SE estimate, standard error of estimated value by the equation, n = 65.
viability, is not likely to influence female RNA levels as eggs are fertilized shortly before their release. This, however, requires further investigation as in the absence of re-mating, egg production and egg viability often decrease, albeit at different rates (Gorokhova, 2010). Moreover, if poor fertilization success is related to the fertilization capacity of the male sperm, as shown for the calanoid copepod Temora stylifera (Ianora et al., 1999), the low egg viability is unlikely to be related to female RNA content. Using national zooplankton monitoring abundance data from the study area, we attempted to correlate the percentage of males in the A. tonsa population (range 0–67%) to the EPR values and %Viability obtained in our experiments and found no significant correlations ($r = -0.25$ and $r = 0.06$, respectively; $P > 0.5$ in both cases), suggesting that lack of fertilization was not a contributing factor.

To summarize, in wild-caught A. tonsa females, individual RNA content is positively related to egg production irrespective of the egg viability status. Hence, RNA-based indices, while being suitable proxies for individual in situ growth assessment, might not be applicable for copepod recruitment studies when the proportion of viable eggs is low or fluctuates widely. In such cases, this proxy should be combined with egg viability assessments using egg viability assays or egg hatching experiments.

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REFERENCES


