Vol. 407: 87–98, 2010
doi: 10.3354/meps08540

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published May 20

Seasonal and spatial variations in the RNA:DNA ratio and its relation to growth in sub-Arctic scallops

Martin Emil Blicher[1,3,*], Catriona Clemmesen[2], Mikael Kristian Sejr[3], Søren Rysgaard[1]

[1]Greenland Climate Research Centre, Greenland Institute of Natural Resources, Kivioq 2, Box 570, 3900 Nuuk, Greenland
[2]Leibniz Institute of Marine Sciences (IFM-GEOMAR), Düsternbrooker Weg 20, 24105 Kiel, Germany
[3]National Environmental Research Institute, Aarhus University, Vejlsøvej 25, 8600 Silkeborg, Denmark

ABSTRACT: We examined the RNA and DNA concentration of field-caught scallops *Chlamys islandica*, maintained in suspended cultures at 15 and 30 m depth, and scallops from a wild population at 50 to 60 m in Kobbefjord, southwest Greenland. General relations between RNA and DNA concentrations and individual shell height were established, and we found that the RNA:DNA ratio (RD) worked well as a standardisation of the RNA concentration independent of size and sex. During an experimental period of 14 mo, we observed a pronounced seasonal pattern in RD and mass growth, and differences between depths. Even though the period with high levels of RD reflected the growth season relatively well, RD was a poor predictor of individual mass growth rates of *C. islandica*. However, we found a non-linear response in RD to increased food concentrations resulting in RD being up- and down-regulated at the beginning and end of the productive summer season, respectively. These results indicate that short-term dynamics in the actual mass growth rate might be controlled through regulation of ribosome activity rather than ribosome number (RNA concentration). This adaption would allow scallops to up-regulate protein synthesis more rapidly, thereby ensuring efficient utilisation of the intense peaks in food availability in coastal areas in the Arctic. Therefore, we suggest that RD in *C. islandica* reflects the growth potential rather than the actual growth rate. Still, the amount of unexplained variance in RD is considerable and not independent over time, suggesting the existence of unresolved mechanisms or relationships.

KEY WORDS: Macrobenthos · Bivalve · Pectinid · *Chlamys islandica* · Greenland · Biomarker · Food availability

INTRODUCTION

Bivalves dominate shallow benthic communities in the Arctic and are considered to have a large functional importance in the marine ecosystem in coastal areas (Sejr et al. 2000, 2007, Blicher et al. 2009). Not only do they affect the physical and biochemical characteristics of the benthic habitat (Graf & Rosenberg 1997, Ragnarsson & Raffaelli 1999, Riisgård & Larsen 2005), but they are also important sources of food for several dominant predators, i.e. eider duck, long-tailed duck, walrus, bearded seal, cod, and wolf fish (Liao & Lucas 2000, Link & Garrison 2002, Born et al. 2003, Dehn et al. 2007, Merkel et al. 2007). Hence, the population dynamics of bivalves are expected to affect other trophic levels both directly and indirectly.

Individual production of bivalves in Greenland varies considerably on different temporal and spatial scales (seasonally, inter-annually, along depth gradients, and geographically; Sejr et al. 2009, Blicher et al. in press). However, traditional approaches to studying growth variation in bivalves (cohort analysis, tag-recapture, annual growth increments) are time-consuming and elaborate. This is further complicated by the logistical challenges in Greenland, which spans from 60 to 84° N in its geographical range (~2700 km)

*Email: mabl@natur.gl

with a pronounced seasonal sea ice cover. Consequently, little is known about the spatial or the temporal dynamics in the marine environment off Greenland. This is in strong contrast to the need for knowledge about the ecological relationships in the marine environment in the Arctic (ACIA 2005). Therefore, the validation of an easily obtained proxy for the growth rate of Arctic marine fauna would be of great value in studies of population dynamics. It would provide the opportunity to study growth dynamics at a multitude of locations with contrasting habitat characteristics, potentially revealing causal relationships between environment and biota. The RNA:DNA ratio (RD) of animal tissue is a measure of the capacity for protein synthesis (Wright & Hetzel 1985, Dahlhoff 2004). RD has been widely used in studies of fish larvae, where it has been validated as a proxy for the mass growth rate or the physiological condition (Buckley 1984, Clemmesen & Doan 1996, Grønkjær et al. 1997, Caldarone et al. 2003, Buckley et al. 2008). In studies of invertebrate taxa, it has been suggested that variations in nucleic acid ratios (RD, RNA:protein, or total RNA concentration) relate to metabolic activity, physiological robustness, reproductive state, food availability, or temperature (Robbins et al. 1990, Lodeiros et al. 1996, Buckley & Szmant 2004, Dahlhoff 2004, Kim et al. 2005, Norkko et al. 2005a, 2006b, Fraser et al. 2007). Some studies have suggested that RD is affected by size and sex (Robbins et al. 1990, Lodeiros et al. 1996, Roddick et al. 1999, Chicharo et al. 2007). Even though it has often been assumed that RD can be regarded as an indirect measure of mass growth rate, only few studies have measured the actual growth rate of bivalves simultaneously with RD. However, Lodeiros et al. (1996) found that a highly significant positive correlation between growth rate and RD of juvenile scallops in Venezuela weakened in maturing individuals. Altogether, these results indicate that a number of parameters need to be taken into account when interpreting RD variations, and that its suitability as a proxy for the mass growth rate should be critically examined before implementation into research and monitoring programs. Still, it is likely that RD has a large potential in marine ecological studies in polar areas (Norkko et al. 2005a).

The scallop *Chlamys islandica* is common from temperate to high-Arctic areas at depths down to 130 m. It is widely distributed along the coast of Greenland (Pedersen 1994). Studies of growth variation of *C. islandica* in Kobbefjord, SW Greenland, showed that growth differed significantly between depths and seasons, and that these differences were caused primarily by variation in food availability. Thus, we have suggested that *C. islandica* is generally food limited in its natural habitat in SW Greenland (Blicher et al. 2009, in press). In parallel with studying the temporal and spatial growth variation, we took tissue samples for the analysis of RNA and DNA concentration in *C. islandica*.

Here our aim was to describe seasonal variations in RD in the tissue of *Chlamys islandica* at 3 different depths in Kobbefjord. We then compared these data to variations in mass growth rate in order to evaluate the potential of RD as a general proxy for the growth rate of this species. We hypothesised that mass growth rate and RD varied synchronously, revealing a causal relationship. In addition, we examined the possibility of a direct coupling to scallop condition and to variations in food availability and temperature in the surrounding water column.

MATERIALS AND METHODS

Experimental setup. ***Suspended scallops:*** *Chlamys islandica* were collected using a triangular dredge in May 2007 at 50 to 60 m depth in the outer Kobbefjord. To be able to estimate future individual growth rates, we measured the initial shell height (SH) of all scallops to the nearest 0.01 mm and tagged them individually with numbered shellfish tags (4 × 8 × 0.15 mm, Hallprint) on the upper shell. Scallops were divided into 2 different initial size groups, representing immature (15 to 35 mm SH: Group 1) and maturing bivalves (35 to 55 mm SH: Group 2; Pedersen 1994, Blicher et al. 2009), and transferred to lantern box nets (FUKUI type, Coastal Aquacultural Supply). Each of the 2 size groups was suspended in lantern nets at 2 different depths, 15 and 30 m, approximately 1 nautical mile from the site of collection in Kobbefjord. Thus, we analysed 4 separate groups, differing in size and/or deployment depth. Despite individual growth during the experiment, we will keep the separation of individuals into 'size groups 1 and 2' referring to the initial SH of the scallops. During a period of ~14 mo, we measured the shell growth of all individuals at approximately monthly intervals. On 11 occasions, ~10 individuals from each size group and depth were randomly sub-sampled (n = 436 in total).

Wild scallops: In addition to the suspended scallops, we collected wild scallops at 50 to 60 m depth in the outer part of Kobbefjord in the same intervals as the suspended scallops. Again, individuals were separated into size groups 1 and 2, with 10 individuals in each group (n = 216 in total).

Analysis of RNA and DNA concentration. Immediately after collection, we removed a tissue sample from the adductor muscle of each sub-sampled individual for analyses of RNA and DNA concentration. The tissue (10 to 100 mg wet mass) was transferred to a sterile Eppendorf tube and kept at –80°C. During the en-

tire experiment we extracted tissue samples from a total of 652 individuals. During the period from sampling in Greenland until the analysis of RNA and DNA concentration in the laboratory at IFM-GEOMAR, Kiel, Germany, the tissue samples were continuously stored at a temperature below –60°C. They were transported from Greenland by ship (RV 'Dana') to Denmark stored in a –80°C freezer. During transport from Denmark to the laboratory in Kiel (~4 h), samples were kept on dry ice (–60°C). The concentration of nucleic acids in the tissue was analysed using a modification of the method of Clemmesen (1993) and Belchier et al. (2004). Adductor muscle tissue samples were freeze-dried in opened vials for 16 h, using a Christ Alpha 1-4 freeze-drier at –51°C and weighed to the nearest 0.1 µg (Sartorius microbalance SC2). From the total freeze-dried sample, a subsample with an approximate dry weight of 0.5 mg was cut out and transferred to a new vial. Three large (∅ 2 mm) and a spatula tip of small (∅ 0.2 mm) glass beads and 400 µl Tris-EDTA extraction buffer (Tris 0.05 M, NaCl 0.01M, EDTA 0.01M) containing a detergent (SDS 0.01%) were added. Rehydration took place on ice for 30 min. Cells were disrupted by shaking in a cell mill (Mixer Mill MM2, Retsch) for 15 min. The homogenate was centrifuged for 8 min at 3829 × *g* (6800 rpm) and 0°C (Sigma Laboratories Centrifuges 3-18K). The supernatant (300 µl) was pipetted into a new 1.5 ml cap vial, diluted according to the dry weight of the sample, and vortexed, and 130 µl were pipetted into a black 96 well microtitre plate. For each measurement, calibration curves for RNA and DNA were determined ($r^2 > 0.98$), and a control homogenate was added. The fluorometric assay was performed on a Labsystems Fluoroscan Ascent using integrated dispensers for both the nucleic acids stain (ethidium bromide) and buffer (Tris-EDTA), with an excitation wavelength of 355 nm and measuring at an emission wavelength of 590 nm. Autofluorescence was measured first, before the fluorophore ethidium bromide was added. Subsequently, total nucleic acid fluorescence was measured, and RNase (Serva, Ribonuclease A, 34388) was added to degrade the RNA. After the enzyme treatment (30 min at 37°C), the remaining (DNA) fluorescence was measured. RNA fluorescence was calculated by subtracting DNA fluorescence from the total nucleic acid fluorescence. RNA was calculated based on the standard curve using 16S, 23S ribosomal RNA (Boehringer Mannheim, order no. 10206938001). The DNA concentrations were calculated using the relationship between RNA and DNA fluorescence described by Le Pecq & Paoletti (1966) using a slope ratio of 2.2 for DNA to RNA.

Scallop mass growth rate and condition. Scallops sampled for the analyses of nucleic acids and biomass were dissected into gonad and somatic tissues. The sex was determined by the colour of the gonads. We measured the wet mass and determined dry mass (DM) by drying at 60°C for 72 h or until they reached a constant weight. In the following text we refer to condition and mass growth in terms of total tissue mass.

Blicher et al. (2010) obtained a general relation between SH (mm) and individual DM (g) of *Chlamys islandica*:

$$DM_{pred} = 4.86 \times 10^{-6}\ SH^{3.269} \quad (n = 502,\ R^2 = 0.94,\ p < 0.001) \tag{1}$$

from which we calculated a biomass index (BMI) for each individual collected:

$$BMI = DM_{obs}/DM_{pred} \tag{2}$$

For the suspended (and tagged) scallops, a combination of individual shell growth rates and changes in BMI were used to estimate the individual instantaneous mass growth rate, G_m (d^{-1}) of scallops between 2 sampling dates:

$$G_{m(1,2)} = \frac{\mathrm{Ln}\left[\dfrac{DM_{obs(2)}}{DM_{pred(1)} \times \overline{BMI}_{i(1)}}\right] \times 1000}{\Delta t_{(1,2)}} \tag{3}$$

where DM_{pred} is estimated from the general model, Eq. (1). $\overline{BMI}_{i(1)}$ is the average biomass index at time 1, and the subscript *i* refers to the 4 combinations of depth (15, 30 m) and size group (1, 2). $DM_{obs(2)}$ is the observed dry mass for an individual collected at time 2.

We also estimated an index of condition similar to what has been termed a 'gravimetric index of condition', CI, by Norkko et al. (2005b), which is the tissue dry mass to wet mass ratio (CI = DM:WM). Contrary to other biomass indices relating tissue DW to shell weight or height, CI is independent of a potential asynchrony in the growth of tissue and shell. The index is suggested to reflect tissue gain or loss within days to months (Norkko et al. 2005b). The mass of the muscle tissue used for RNA and DNA analysis was included in the calculation of CI and mass growth rate.

Environmental parameters. Temperature was registered every 6 h at 15 and 30 m depth throughout the experimental period using temperature loggers attached to the lantern nets (HOBO U22 Water Temp Pro v2, Onset Computer Corporation). At 55 m depth, temperature was registered with a CTD (SBE 19+, Sea-Bird Electronics). Approximately every second week, and always on the days of scallop sampling, we took water samples with a Niskin-type sampler (KC-Denmark, Silkeborg) for the analysis of photosynthetic pigments and particulate carbon at the specific sites and depths.

Seawater samples from 15, 30 and 55 m depth were filtered (Whatman GF/C, <0.2 bar) for determination of chlorophyll *a* (chl *a*). The filters were extracted in 96%

ethanol for 18 h in the dark. After extraction, the samples were analysed on a Turner Designs TD-700 fluorometer. Chl *a* in the samples was calculated according to Parsons et al. (1984).

Total particulate carbon (TPC) was measured on water samples filtered onto Whatman GF/C filters. After filtration, the samples were dried at 60°C for 24 h and stored separately until analysis on an elemental analyser (ANCA-GSL, SerCon).

Statistical analyses. We examined the reliability of RD as a general proxy for the mass growth rate, G_m, by linear regression using data from all individuals collected. However, the instantaneous mass growth rate was assumed to be size dependent. Also, results from studies of fish larvae indicate that temperature can affect the relation between growth and RD due to the effect of temperature on the turnover of RNA (e.g. Buckley et al. 2008). To test this, the relation between G_m and the independent variables RD, SH and water temperature on the day of scallop sampling was examined by multiple linear regression analysis. Residuals were tested for autocorrelation structure (Lag = 1). We used R-square and the Akaike Information Criterion (AIC) to compare the fit of the models.

The relations between the average RD and environmental parameters, TPC, chl *a*, and temperature, respectively (measured on the day of scallop sampling), were examined by non-linear regression using a maximum likelihood estimation method taking heteroscedasticity into account (Blicher et al. 2007). We used either a logistic function or the Gompertz function, depending on which gave the best fit of data:

Logistic: $$f(x) = \frac{A_1 - A_2}{1 + (x/x_0)^p} + A_2 \quad (4)$$

Gompertz: $$f(x) = A e^{-\exp(-K(x - x^*))} \quad (5)$$

In the logistic function, A_1 is the initial value, A_2 is the asymptotic value, x_0 is the centre and p is the power. In the Gompertz function, A is the amplitude, x^* is the centre and k is a coefficient. Residuals from the best fit model were examined for any trends.

RESULTS

RNA and DNA concentration in relation to size and sex

RNA and DNA concentration in *Chlamys islandica* was size dependent, decreasing with increasing SH. This was fitted with power functions (RD = aSH^b) revealing identical effects of SH (mm) on RNA and DNA concentration (µg mg DM^{-1}), respectively (b ~ –0.5; Fig. 1a,b):

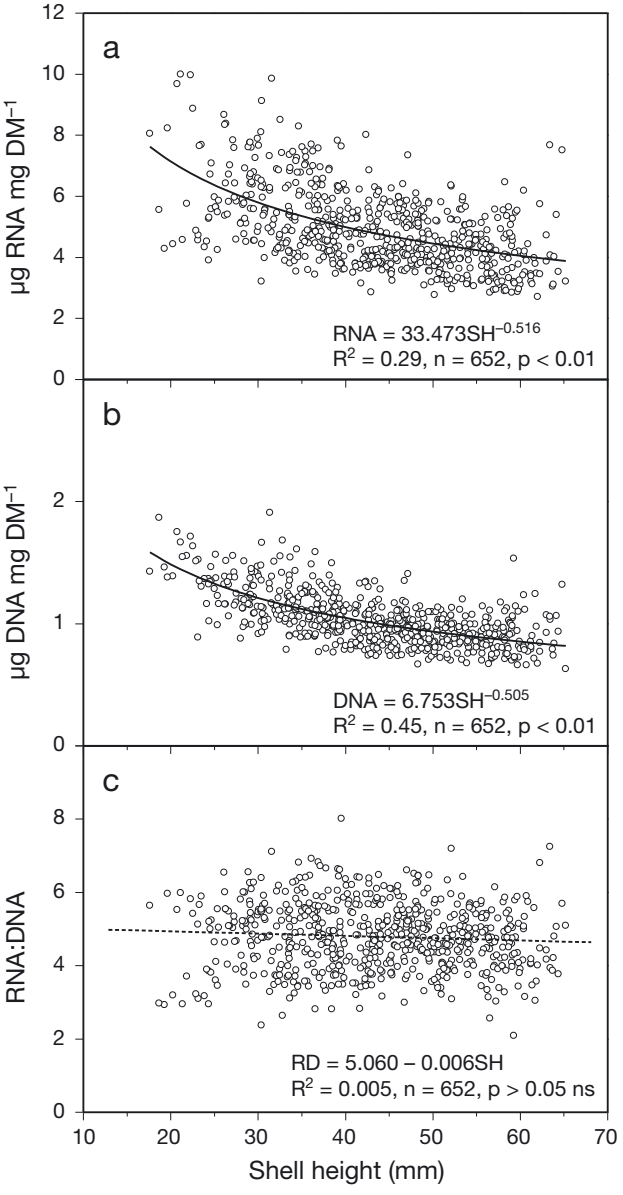

Fig. 1. *Chlamys islandica*. Plots of the effect of shell height on (a) RNA concentration, (b) DNA concentration and (c) RNA:DNA ratio (RD) in the adductor muscle. Plots include data from suspended cultures at 15 and 30 m depth, and from scallops from a wild population in Kobbefjord collected at 50 to 60 m depth. DM: dry mass; SH: shell height

$$\text{RNA} = 33.473\,\text{SH}^{-0.516} \quad (R^2 = 0.29,\ n = 652,\ p < 0.01)$$

$$\text{DNA} = 6.753\,\text{SH}^{-0.505} \quad (R^2 = 0.45,\ n = 652,\ p < 0.01)$$

The RNA concentrations ranged from 3 to 10 µg RNA mg DM^{-1}, with a general decrease from ~8 µg RNA mg DM^{-1} in the smallest individuals to ~4 µg RNA mg DM^{-1} in the largest scallops examined. The DNA concentrations ranged from 0.6 to 2 with an average of ~1.6 and ~0.8 in small and large scallops, respectively. However, there was no trend in RD plotted against individual SH

($p > 0.05$), and the average RD was ~5, ranging from a maximum of ~8 to a minimum of ~2 (Fig. 1c). Sex had no significant effect on individual RD (1-way analysis of variance [ANOVA], $F_{1,507} = 1.08$, $p = 0.29$).

Seasonal and spatial variation in RD

We observed a clear seasonal pattern in the level of RD at all 3 depths (Figs. 2 & 3). This pattern was characterised by a high level during summer (May to September/October), decreasing through autumn and reaching a minimum in the winter months (January to April) followed by an abrupt increase in spring (April to May). The pattern was evident for both size groups. On the spatial level, the variation was almost identical at 15 and 30 m depth. No consistent differences between these 2 depths were observed until the last sampling date (Fig. 3). However, RD values in scallops collected from the wild population at 50 to 60 m depth were con-

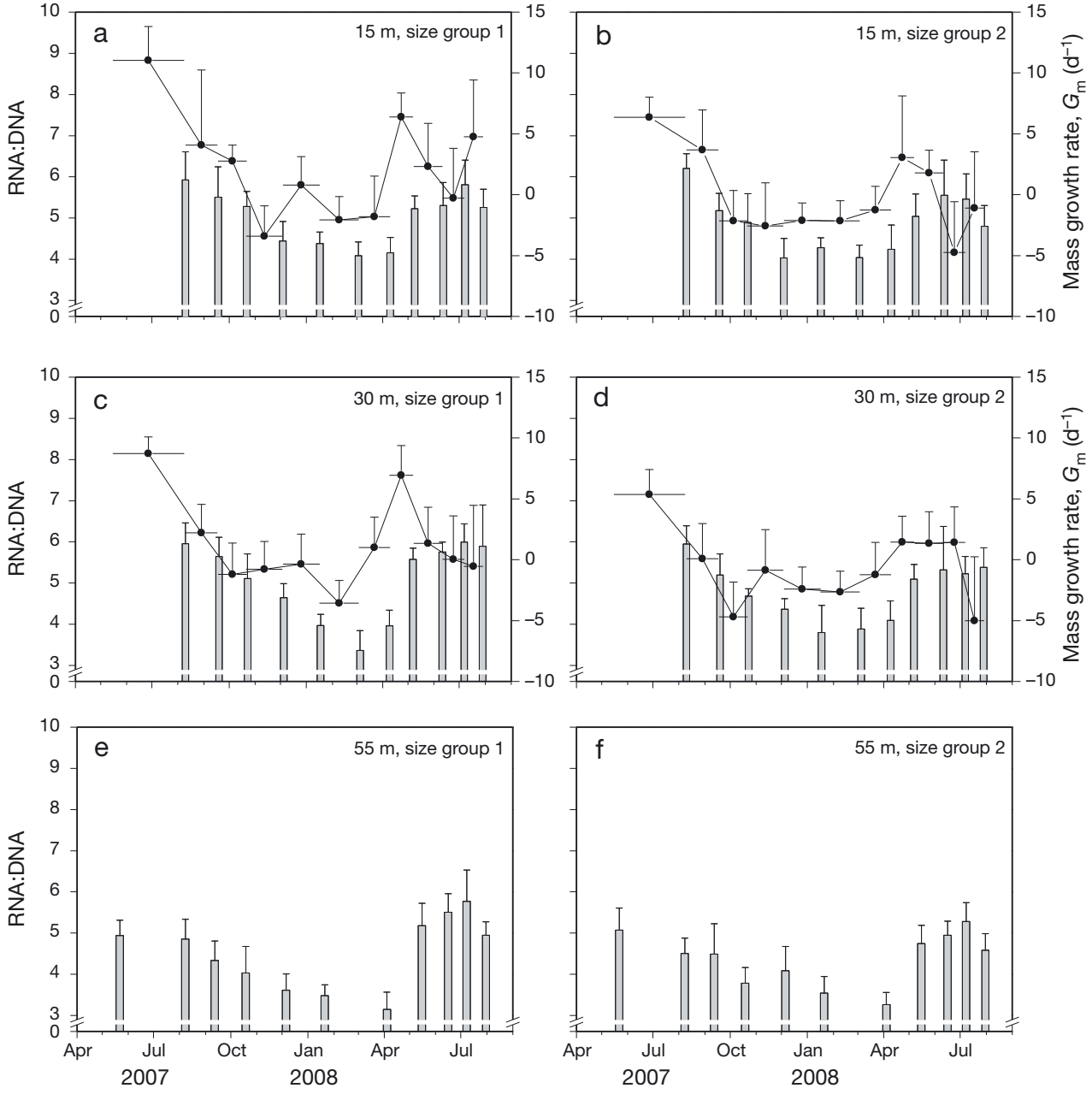

Fig. 2. *Chlamys islandica*. Seasonal variation in the average RNA:DNA ratio (bars) and the instantaneous mass growth rate, G_m (-●-), during 2007 and 2008 at (a,b) 15 m, (c,d) 30 m and (e,f) 50 to 60 m in Kobbefjord. Vertical error bars indicate the standard deviation (n = 10). Horizontal error bars for G_m indicate the period for which the average values were estimated

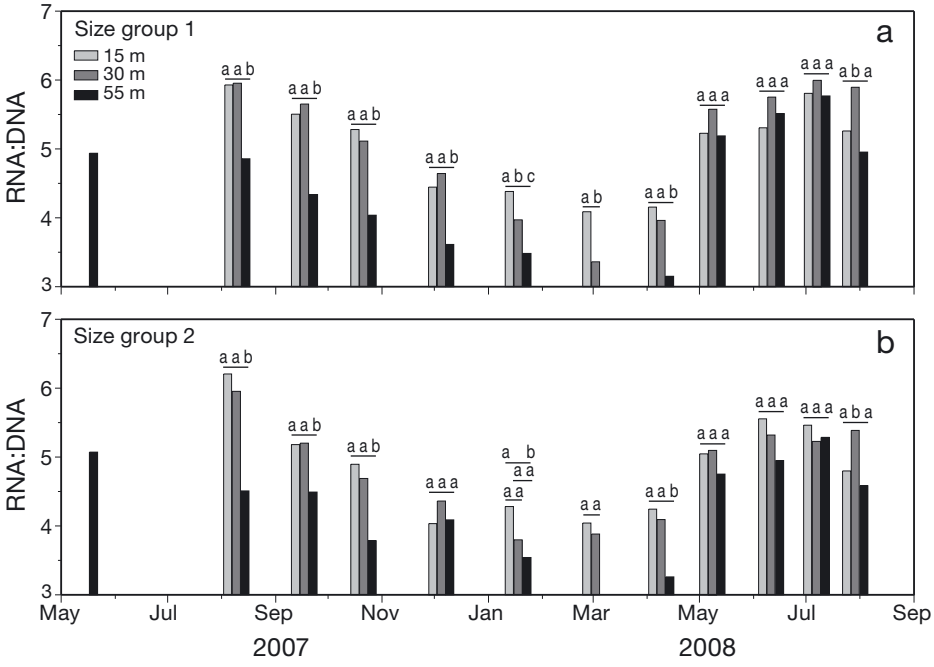

Fig. 3. *Chlamys islandica*. Comparison of the seasonal variation in average RNA:DNA ratio (RD; n = 10) at 15, 30 and 55 m depth in size groups 1 and 2, respectively. Different letters indicate significant differences in RD (1-way ANOVA, $p < 0.05$) between depths on the given sampling dates

sistently lower than in the suspended scallops from the beginning of the experiment until April 2008. During spring 2008, the RD level increased abruptly, reaching a level similar to that at the 2 shallower depths. This lasted throughout the period from May to the end of July, when the experiment was terminated (Fig. 3).

Coupling of RD to mass growth rate

As in the case of RD, we found a clear seasonal signal in the instantaneous mass growth rate of scallops in the suspended cultures at 15 and 30 m depth. We observed peak growth in spring and early summer, and zero growth or slightly negative growth rates during winter (Fig. 2a–d). A simple linear regression between individual G_m and RD gave a significant positive correlation ($R^2 = 0.19$, $p < 0.01$, n = 436; Fig. 4a, Table 1). Although significant, the correlation between CI and RD was even poorer ($R^2 = 0.11$, $p < 0.01$, n = 652; Fig. 4b), and therefore we only report results on G_m in the further presentation of data. Adding SH as a second independent variable resulted in an increase in the model fit (Table 1, Model II, AIC = 2452.4, $R^2 = 0.26$, n = 436), indicating a significant negative effect of increasing SH on the instantaneous mass growth rate of scallops ($p > 0.01$), which can be regarded as a correction for size dependence in G_m. Neither temperature nor the interaction term RD × Temp had any significant effect ($p > 0.05$) and increased the AIC (models not shown). However, to examine the robustness of the results to the fact that data were sampled at different depths,

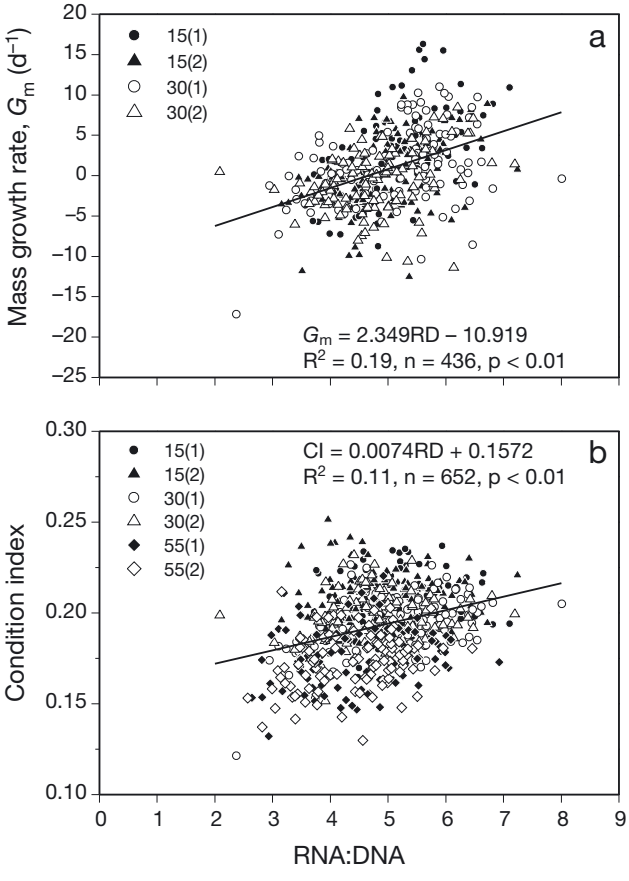

Fig. 4. *Chlamys islandica*. Simple linear regressions of the effect of individual RNA:DNA ratio (RD) on (a) mass growth rate, G_m, and (b) condition index, CI. Different depths (15, 30 and 55 m) and size groups (1 and 2) are illustrated by different symbols

Table 1. *Chlamys islandica*. Coefficients (±SE) and Akaike information criterion (AIC) of the regression models for the effect of RNA:DNA ratio (RD) and shell height on the instantaneous mass growth rate (G_m) (n = 436). To examine the robustness of the results, dummy variables for depth were added to the model, and we tested for autocorrelation structure of the residuals. Non-significant parameters (ns, $p > 0.05$) were backward eliminated (Temperature and RD × Temp). $^{*}p < 0.05$, $^{**}p < 0.01$

	Model			
	I	II	III	IV
Intercept	−10.919** (1.172)	−4.120** (1.540)	−4.409** (1.537)	−4.409** (1.537)
RNA:DNA	2.349** (0.232)	2.114** (0.225)	2.106** (0.224)	2.106** (0.224)
Shell height (mm)	–	−0.127** (0.012)	−0.129** (0.020)	−0.129** (0.020)
Control variables				
15 m	–	–	0.865* (0.383)	0.865* (0.383)
30 m	–	–	0.000	0.000
Auto correlation				
Lag 1	–	–	–	−0.403** (0.045)
R^2	0.191	0.262	0.270	0.385
AIC	2490.3	2452.4	2443.2	2372.6

dummy variables for depth were added to the model. Even though the model fit was only slightly improved, the result indicated significant spatial differences ($p = 0.024$) in the relation between G_m and RD (Table 1, Model III, AIC = 2443.2, $R^2 = 0.27$, n = 436). Moreover, there was a significant autocorrelation structure of the residuals of this model (Lag 1, $p < 0.01$) as shown in Model IV (AIC = 2372.6, $R^2 = 0.385$, n = 436).

Given the observed decline in the actual RNA concentration with increasing size (Fig. 1a), we found it intuitive to test whether the actual RNA concentration was a better predictor of the mass growth rate compared to the standardised (size-independent) parameter, RD. Therefore, we repeated the analytical approach with RNA concentration as the explanatory parameter. Although the RNA concentration correlated positively to G_m, it gave a slightly poorer fit ($R^2 = 0.17$, $p < 0.01$, n = 436). The model was not improved by adding other predictors, and also in this model, we found a significant autocorrelation structure of the residuals.

Coupling of RD to environmental parameters

The annual temperature cycle shows an amplitude ranging from –1 to –1.5°C in February and March, with similar temperatures at all depths, to 4.5 to 6°C at 15 m depth and 3 to 4°C at 30 and 55 m in late summer and early autumn, respectively. The concentration of TPC in Kobbefjord ranged between ~0.06 and ~0.5 mg l^{-1} in winter and spring/summer, respectively, within the depth range studied. Peaks were observed in the spring of both 2007 and 2008. Overall, a seasonal cycle could be separated into 2 distinct periods; a period from mid-April to October with relatively high levels of TPC (0.10 to 0.5 mg l^{-1}), and a period from November to April during which TPC was stable in the range 0.06 and 0.10 mg l^{-1} at all 3 depths. However, in the spring/summer season there was a depth gradient in TPC showing decreasing values of TPC with increasing depth, generally amounting to a factor of 2 in difference in the depth range studied. A similar pattern was observed for chl *a*, although concentrations were more variable throughout the spring and summer period. A phytoplankton spring bloom was observed in May 2007 and 2008, where chl *a* concentrations peaked at 1.5 to 3 µg l^{-1} followed by declining concentrations at all 3 depths. An intense late summer bloom occurred at the end of August 2007 and in late July 2008 at 15 m (chl *a* up to 5 µg l^{-1}), while chl *a* concentrations at 30 and 55 m declined continuously during late summer to a minimum of ~0.01 µg l^{-1} during winter.

When analysing the relationship of RD to TPC, chl *a* and temperature, TPC was the better predictor of RD following a sigmoid response curve (logistic function):

$$RD = \frac{-5.709}{1+(TPC/0.050)^{2.952}} + 5.368$$

($R^2 = 0.62$, n = 66, $p < 0.01$)

corresponding to $A_1 = -0.341$, $A_2 = 5.368$, $x_0 = 0.050$ and $p = 2.958$ in Eq. (4). RD seemed to respond rapidly to small increases in food level until reaching a maximum at ~0.15 mg TPC l^{-1}, after which RD reached an upper level where it was independent of food concentrations (Fig. 5a). Using chl *a* as an explanatory parameter revealed a similar pattern, which was best fitted with a Gompertz function (Eq. 5):

$$RD = 5.143e^{-\exp(-11.174(CHL+0.996))}$$

($R^2 = 0.41$, n = 66, $p < 0.01$)

However, this equation explained less of the variance in RD than TPC (Fig. 5b). RD displayed a weak but significant correlation to temperature when fitted with a Gompertz function (Fig. 5c):

$$RD = 5.498e^{-\exp(0.359(TEMP+4.105))}$$

($R^2 = 0.34$, n = 66, $p < 0.01$)

The residuals from the best fit model (TPC as the explanatory parameter, $R^2 = 0.62$) were examined for any

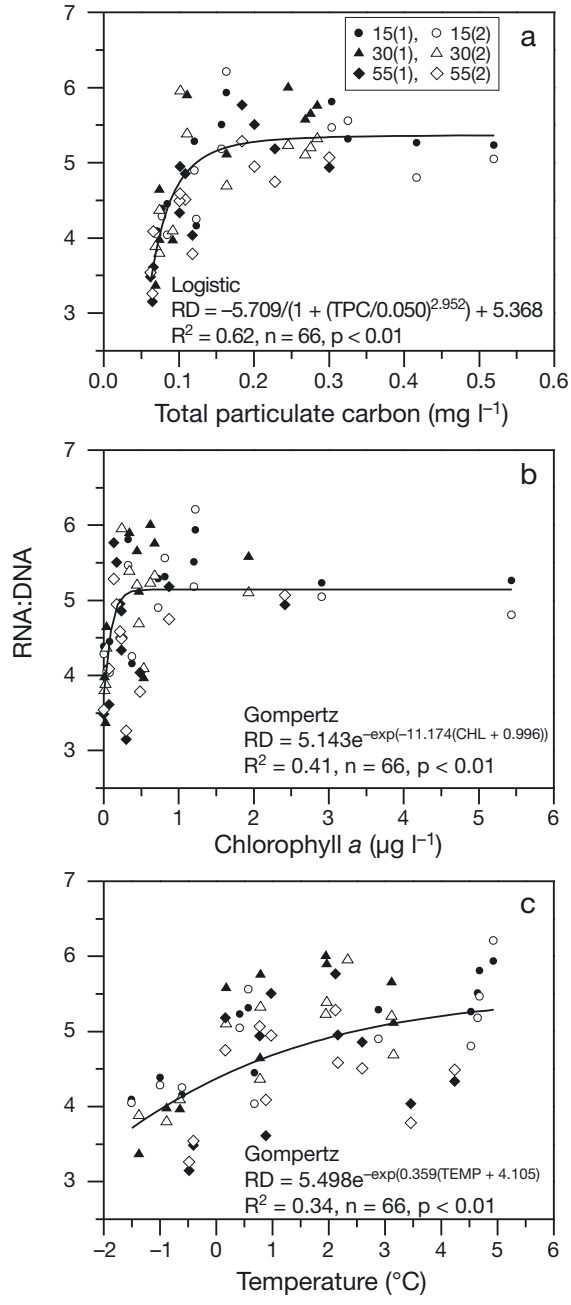

Fig. 5. *Chlamys islandica.* Plots of environmental parameters (a) total particulate carbon (TPC), (b) chlorophyll *a* and (c) temperature against the average RNA:DNA (RD) ratio. Depths (15, 30 and 55 m) and size groups (1 and 2) are illustrated by different symbols. Data are fitted with non-linear models

trends, but we found no significant correlation of the residuals to either chl *a* or temperature ($p > 0.05$). Nevertheless, a plot of the residuals against time indicates that the residuals of the model were not independent over time, and that the model could not explain all of the observed spatial differences in RD (Fig. 6).

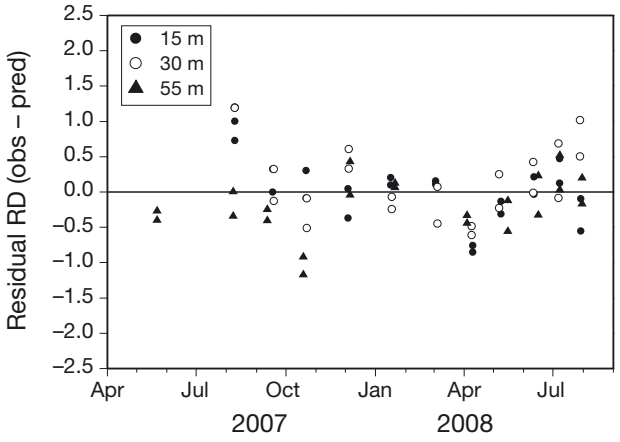

Fig. 6. Residuals ($RD_{observed} - RD_{predicted}$, where RD is the RNA:DNA ratio) of the best fit model (total particulate carbon as the explanatory parameter, Fig. 5a) plotted against time. Depths are indicated by different symbols

DISCUSSION

Effects of size on RNA and DNA concentration

RNA and DNA concentration in *Chlamys islandica* was size dependent, decreasing with increasing SH (Fig. 1a,b). Size dependence of RNA concentration has been observed in other studies (Norkko et al. 2005a, Norkko & Thrush 2006). Norkko & Thrush (2006) proposed that the RNA concentration would reach an asymptotic value in the largest size classes of the cockle *Austrovenus stutchburyi*, and suggested that this value represents the basic metabolism required for vital protein synthesis. In the present study, we focused on size classes with significant somatic growth potential, i.e. young specimens much smaller than the maximum SH of *C. islandica* (80 to 110 mm) (Pedersen 1994, Blicher et al. 2009). Thus, we could not completely validate the existence of a baseline value of RNA concentration for this species. However, our data seem to approach a minimum value of ~3 µg RNA mg DM^{-1} with increasing SH. It has been argued that the RNA concentration in polar organisms is high compared to warmer areas as a compensation for generally lower translation efficiencies at cold temperatures, or due to slower RNA turnover (Fraser et al. 2002, Caldarone et al. 2003, Norkko et al. 2005a, Storch et al. 2005, Clarke 2008). However, this is difficult to evaluate due to species-specific variations and differences in the analytical procedure between studies.

The RNA concentration in itself has been used as a biomarker in some studies due to inconsistent patterns in DNA concentration, which prevented its use for standardising RNA concentration using RNA/DNA ratios (e.g. Norkko et al. 2005a). It is evident from our

results and the results of others that the use of RNA concentration as a biomarker should include a test for size dependence, or studies should concentrate on individuals within a very narrow size range in order to prevent any bias of the data.

In this study, we successfully standardised RNA data using DNA concentration. The RD ratio was independent of size, which is in contrast to findings in other bivalve studies of a negative relationship between RD and size (e.g. Lodeiros et al. 1996, Roddick et al. 1999), but consistent with studies of *Crassostrea virginica* and *Mya arenaria* (Pease 1976, Mayrand et al. 1994). RD was also unaffected by the sex of *Chlamys islandica*, unlike what was found for 3 marine species collected off Portugal (Chicharo et al. 2007). Thus, we conclude that RD data of *C. islandica* in SW Greenland can be compared directly independently of size and sex. However, this does not necessarily exclude potential differences in the RD dynamics of scallops at different stages of maturity.

Seasonal and spatial variation in RD

There was a clear seasonal trend in scallop RD, which also varied significantly between depths. The seasonal signal in RD was consistent between size groups. First of all, in combination with the overall seasonal pattern found at the 3 depths, with relatively high values during summer and decreasing RD during autumn and winter until a sharp increase in the spring, these results indicated that the RNA and DNA concentration in scallop muscular tissue could be measured accurately enough to reveal potential differences between habitats and seasons. Therefore, it also seemed reasonable to conclude that this high-resolution dataset qualified to test the potential of RD as a proxy for the mass growth rate, or alternatively, to examine the causal relationships behind the seasonal and spatial variation in the RD of *Chlamys islandica*.

RD and mass growth rate

RD was only weakly correlated to temporal and spatial variations in scallop mass growth rate. Even though we found a pattern of RD variation very similar to what was expected according to our hypothesis, with high ratios during the productive summer, minimum values during winter, and lower RD at deeper compared to shallow depths, our data indicated that RD did not reflect the variation in mass growth rate very accurately, neither on a temporal nor on a spatial scale (Table 1, Models III and IV). First of all, the difference in mass growth rate between scallops at 15 and 30 m depth, respectively, which was most pronounced during late summer, was not reflected in the RD data. Also, during the summer of 2008, the RD of wild scallops at 50 to 60 m was not significantly different from that of cultured scallops, which was unexpected because size-at-age data for scallops collected at the site have indicated relatively slow growth compared to suspended scallops (Blicher et al. 2009, 2010). Moreover, the peaks in mass growth rate during spring and summer could not be identified in the RD, which did not show any large fluctuations from May to August, but rather appeared to have a stable and high level during this period. By including scallop SH, the model fit was improved (Table 1, Model II), indicating that G_m decreases with increasing individual size. This was expected (Blicher et al. 2010), and the inclusion of SH in Model II (Table 1) can be regarded as a simple correction for this size dependence. However, the large fraction of unexplained variation and the significant autocorrelation of the residuals seemed to indicate that RD explained the seasonal cycle in the mass growth rate of *Chlamys islandica* poorly. This is in contrast to the results in studies of fish larvae growth, where RD has been validated as a proxy for the mass growth rate within a period of 2 to 7 d before RD analysis (meta-analysis in Buckley et al. 2008). Also, these studies have suggested that temperature has an effect on the relation between RD and growth, which we did not find. RD and actual growth rates have only been compared directly in marine invertebrates in a few studies. The majority of studies have either assumed that RD or RNA concentration reflects growth or used RD as an indirect measure of condition, nutritional status or metabolic activity (Dahlhoff & Menge 1996, Dahlhoff et al. 2001, Buckley & Szmant 2004, Norkko et al. 2005a). However, Frantzis et al. (1992) also found RD to be inefficient in predicting growth rates of field-collected sea urchins *Paracentrotus lividus*. Lodeiros et al. (1996) concluded that RD provided a good indicator of short-term growth of juvenile scallops *Euvola ziczac*, while being more difficult to interpret in maturing and mature scallops.

There can be several reasons why RD appears to be a poor predictor of the mass growth rate of *Chlamys islandica*: (1) RNA synthesis might be related to metabolic processes other than growth, i.e. mobilisation of muscular energy in support of gametogenesis (Lodeiros et al. 1996). However, the RD variation of immature scallops did not differ from that of maturing scallops in this study. Moreover, variation in RD did not correlate significantly to the overall seasonal variation in a gonad mass index (linear regression, $R^2 = 0.001$, $n = 617$, $p = 0.43$). (2) RD might reflect the general health status rather than growth (e.g. Chicharo & Chicharo 1995); however, our study did not include

other known physiological indicators of stress in order to test this (Dahlhoff et al. 2001, Dahlhoff 2004, Moore et al. 2006). (3) RD and mass growth rate might have been measured on different time scales, which is an important aspect in interpretation of data (Norkko et al. 2006a). G_m is a monthly average, while RD is expected to vary on a scale of days (Dahlhoff 2004). RD also correlated weakly to the condition index, CI (Fig. 4b), which is suggested to reflect gains or losses in weight within days to months (Norkko et al. 2005b). Still, at this stage we cannot reject that differences in the time-scale of our estimates might have had an effect on the results of this analysis. (4) It is also possible that the RNA concentration does not reflect the actual growth rate of *C. islandica* if short-term changes in growth rate are regulated through adjustments in the ribosome activity rather than the quantity. This has been observed to be an important mechanism for adjusting protein synthesis in other animals (Henshaw et al. 1971, Smith et al. 2000). RD would then reflect the potential for growth rather than the actual growth rate.

Despite the possibility that our measurements of RD and mass growth integrate processes occurring on different time scales, our data suggest that RD is a poor predictor of the growth rate of this species and in the given habitat. Still, given the significant seasonal and spatial variation in RD, the questions of what controls the protein synthetic capacity in *Chlamys islandica*, and what it reflects, remain important to our ecological understanding.

Effects of food level on RD

RD correlated significantly to food level (TPC and chl *a*) measured on the day of scallop sampling following a non-linear pattern (Fig. 5a,b). This result indicated that the RNA level in the cells is up-regulated in response to the onset of the phytoplankton growth season, i.e. the shift from winter to spring/summer conditions, but within intermediate to high levels of food concentrations (TPC > 0.15 mg l^{-1}), RD is not affected by changing food levels. This is in accordance with a conceptual model of the dynamics of the RNA response of bivalves to added food, which followed a sigmoid pattern with a species-specific lower and upper level of RNA suggesting that food is the key driver of RD dynamics, often overriding potential negative effects of e.g. hypoxia and increased terrigenous sedimentation (Norkko et al. 2006a,b). Dahlhoff & Menge (1996) and Dahlhoff et al. (2001) also suggested that differences in food conditions are responsible for seasonal and spatial differences in the RD dynamics of molluscs in intertidal systems. However, the residuals of the best fit model in our study (TPC as the explanatory parameter, Fig. 5a) appeared not to be independent through time (Fig. 6), indicating that the fraction of unexplained variation in RD could be due to some forcing or mechanism not monitored in this study. A mechanism allowing faster up-regulation of RNA in response to favourable conditions compared to the down-regulation in response to stressful conditions as suggested by Norkko et al. (2006b) might lead to the observation of higher RD than expected during periods with decreasing food levels. Also, the general individual health status can affect the ability to respond to changing conditions (Norkko & Thrush 2006). Therefore, the response to a key environmental driver is likely to be affected by complex interactions on several ecological levels (individual, habitat, population, community and ecosystem). In any case, it is important to realise that the challenge of discovering causal relationships in ecological field studies is very sensitive to the risk of comparing measurements that integrate processes occurring on different temporal and spatial scales, which might add to the fraction of unexplained variation in the dependent parameters.

What do the dynamics in RD reflect?

The up-regulation of RD in *Chlamys islandica* during spring and summer followed by a down-regulation to winter conditions was a general feature, which matched the growth season with relatively high food levels well. However, from the given experimental setup, it is difficult to evaluate the relative importance of an underlying endogenous rhythm in RD compared to a direct response to environmental drivers. Still, based on the observed pattern, we suggest that the RD ratio primarily reflects the potential for growth in *C. islandica*. The actual growth rate would then vary through changes in RNA activity within the range set by ribosome quantity (RNA concentration). Two general mechanisms could operate to alter the protein-synthesising activity of the ribosomes, one controlling the fraction of ribosomes associated with mRNA (polyribosomes), the other regulating the synthesising activity of polyribosomes. Generally, the ribosomes are able to respond to changes in nutritional condition within hours by changing their activity, while the ribosome quantity is expected to be able to change within days (Henshaw et al. 1971, Millward et al. 1973, 1976, Houlihan et al. 1988, Fraser et al. 2002, Dahlhoff 2004). This would explain the large range of mass growth rates obtained at similar levels of RD as observed for *C. islandica* during spring and summer, as well as the fact that higher growth rates were obtained at 15 m depth compared to 30 m depth at identical RD levels. A high and stable level of RD throughout the spring/

summer would ensure a high capacity for growth in this period. The adaptation might have evolved to ensure an efficient utilisation of the short and intense, but somewhat sporadically occurring summer phytoplankton blooms in the Arctic and in coastal areas in general. Likewise, the winter minimum in RD might reflect a down-regulation of the growth potential in order to minimise metabolic costs in a period of continuously low food availability. This strategy has been observed in other marine invertebrates in polar areas (e.g. Brockington 2001, Brockington et al. 2001).

CONCLUSIONS AND IMPLICATIONS FOR FUTURE RESEARCH

We successfully analysed the RNA and DNA concentration in the tissue of *Chlamys islandica*, and our results revealed clear seasonal and spatial trends in the RNA:DNA ratio (RD), which worked well as a standardisation of the RNA concentration independent of size and sex. Even though the period with high levels of RD reflected the growth season relatively well, the quantity of RNA in the tissue of *C. islandica* was not a reliable proxy for the seasonal variation in mass growth rate of this species. A simple coupling between RD and mass growth rate was presumably obstructed by mechanisms that allowed fast regulation of ribosome activity instead of ribosome number (RNA concentration). Still, our data indicate that RD dynamics depend primarily on the same environmental parameter as the mass growth rate itself, namely food concentration. RD was sensitive to changing food levels from low to intermediate concentrations, resulting in RD being up- and down-regulated in response to the beginning and end of the productive summer season. Based on this relationship, we suggest that *C. islandica* has a higher potential for growth than obtained under the present conditions in SW Greenland, and that the inherent high capacity for growth during spring and summer is an adaptation that makes it possible to up-regulate protein synthesis and thus rapidly ensure efficient utilisation of intense peaks in food availability. The pattern of the unexplained variation in RD indicates the existence of unresolved mechanisms or relationships, and future research should especially concern the temporal scale over which measurements of nucleic acids integrate, and the possible existence of an underlying seasonal endogenous rhythm in order to improve our understanding of the causal relationships.

Acknowledgements. We thank L. Heilman and F. Heinrich for technical assistance, W. Martinsen for laboratory assistance, H. Mempel for help with the tissue nucleic acid analysis, and A. Haxen for linguistic corrections. The authors were financially supported by The Commission for Scientific Research in Greenland (KVUG), The Ministry of Culture, Education and Churches (KIIP) and the Aage V. Jensen Charity Foundation. The project has received financial support from the Danish Energy Agency as part of the climate support programme to the Arctic and is a contribution to the Nuuk Basic Programme.

LITERATURE CITED

ACIA (2005) Arctic Climate Impact Assessment. Cambridge University Press, Cambridge

Belchier M, Clemmesen C, Cortés D, Doan T, Folkvord AG and others (2004) Recruitment studies: manual on precision and accuracy of tools. ICES Tech Mar Environ Sci 33

Blicher ME, Rysgaard S, Sejr MK (2007) Growth and production of sea urchin *Strongylocentrotus droebachiensis* in a high-Arctic fjord, and growth along a climatic gradient (64 to 77° N). Mar Ecol Prog Ser 341:89–102

Blicher ME, Sejr MK, Rysgaard S (2009) High carbon demand of dominant macrozoobenthic species indicates their central role in ecosystem carbon flow in a sub-Arctic fjord. Mar Ecol Prog Ser 383:127–140

Blicher ME, Rysgaard S, Sejr MK (2010) Seasonal growth variation in *Chlamys islandica* (Bivalvia) from sub-Arctic Greenland is linked to food availability and temperature. Mar Ecol Prog Ser 407:71–86

Born E, Rysgaard S, Ehlmé G, Sejr M, Acquarone M, Levermann N (2003) Underwater observations of foraging free-living Atlantic walruses (*Odobenus rosmarus*) and estimates of their food consumption. Polar Biol 26:348–357

Brockington S (2001) The seasonal energetics of the Antarctic bivalve *Laternula elliptica* (King and Broderip) at Rothera Point, Adelaide Island. Polar Biol 24:523–530

Brockington S, Clarke A, Chapman A (2001) Seasonality of feeding and nutritional status during the Austral winter in the Antarctic sea urchin *Sterechinus neumayri*. Mar Biol 139:127–138

Buckley LJ (1984) RNA-DNA ratio: an index of larval fish growth in the sea. Mar Biol 80:291–298

Buckley BA, Szmant AM (2004) RNA/DNA ratios as indicators of metabolic activity in four species of Caribbean reef-building corals. Mar Ecol Prog Ser 282:143–149

Buckley LJ, Caldarone EM, Clemmesen C (2008) Multi-species larval fish growth model based on temperature and fluorometrically derived RNA/DNA ratios: results from a meta-analysis. Mar Ecol Prog Ser 371:221–232

Caldarone EM, Onge-Burns JMS, Buckley LJ (2003) Relationship of RNA/DNA ratio and temperature to growth in larvae of Atlantic cod *Gadus morhua*. Mar Ecol Prog Ser 262: 229–240

Chicharo LMZ, Chicharo M (1995) The DNA/RNA ratio as a useful indicator of the nutritional condition in juveniles of *Ruditapes decussatus*. Sci Mar 59:95–101

Chicharo M, Amaral A, Morais P, Chicharo L (2007) Effect of sex on ratios and concentrations of DNA and RNA in three marine species. Mar Ecol Prog Ser 332:241–245

Clarke A (2008) Ecological stoichiometry in six species of Antarctic marine benthos. Mar Ecol Prog Ser 369:25–37

Clemmesen C (1993) Improvements in the fluorometric determination of the RNA and DNA content of individual marine fish larvae. Mar Ecol Prog Ser 100:177–183

Clemmesen C, Doan T (1996) Does otolith structure reflect the nutritional condition of a fish larva? Comparison of otolith structure and biochemical index (RNA/DNA ratio) determined on cod larvae. Mar Ecol Prog Ser 138:33–39

Dahlhoff EP (2004) Biochemical indicators of stress and metabolism: applications for marine ecological studies. Annu Rev Physiol 66:183–207

Dahlhoff E, Menge B (1996) Influence of phytoplankton concentration and wave exposure on the ecophysiology of *Mytilus californiensis*. Mar Ecol Prog Ser 144:97–107

Dahlhoff EP, Buckley BA, Menge BA (2001) Physiology of the rocky intertidal predator *Nucella ostrina* along an environmental stress gradient. Ecology 82:2816–2829

Dehn LA, Sheffield GG, Follmann EH, Duffy LK, Thomas DL, O'Hara TM (2007) Feeding ecology of phocid seals and some walrus in the Alaskan and Canadian Arctic as determined by stomach contents and stable isotope analysis. Polar Biol 30:167–181

Frantzis A, Gremare A, Vetion G (1992) Growth rates and RNA-DNA ratios in *Paracentrotus lividus* (Echinodermata, Echinoidea) fed on benthic macrophytes. J Exp Mar Biol Ecol 156:125–138

Fraser KPP, Clarke A, Peck LS (2002) Low-temperature protein metabolism: seasonal changes in protein synthesis and RNA dynamics in the Antarctic limpet *Nacella concinna* Strebel 1908. J Exp Biol 205:3077–3086

Fraser KPP, Clarke A, Peck LS (2007) Growth in the slow lane: protein metabolism in the Antarctic limpet *Nacella concinna* (Strebel 1908). J Exp Biol 210:2691–2699

Graf G, Rosenberg R (1997) Bioresuspension and biodeposition: a review. J Mar Syst 11:269–278

Grønkjær P, Clemmesen C, St. John M (1997) Nutritional condition and vertical distribution of Baltic cod larvae. J Fish Biol 51:352–369

Henshaw EC, Hirsch CA, Morton BE, Hiatt HH (1971) Control of protein synthesis in mammalian tissues through changes in ribosome activity. J Biol Chem 246:436–446

Houlihan DF, Hall SJ, Gray C, Noble BS (1988) Growth rates and protein turnover in Atlantic cod, *Gadus morhua*. Can J Fish Aquat Sci 45:951–964

Kim SK, Rosenthal H, Clemmesen C, Park KY, Kim DH, Choi YS, Seo HC (2005) Various methods to determine the gonadal development and spawning season of the purplish Washington clam, *Saxidomus purpuratus* (Sowerby). J Appl Ichthyology 21:101–106

Le Pecq JB, Paoletti C (1966) A new fluorometric method for RNA and DNA determination. Anal Biochem 17:100–107

Liao YY, Lucas MC (2000) Growth, diet and metabolism of common wolf-fish in the North Sea, a fast-growing population. J Fish Biol 56:810–825

Link JS, Garrison LP (2002) Trophic ecology of Atlantic cod *Gadus morhua* on the northeast US continental shelf. Mar Ecol Prog Ser 227:109–123

Lodeiros C, Fernandez R, Bonmati A, Himmelman J, Chung H (1996) Relation of RNA/DNA ratios to growth for the scallop *Euvola* (*Pecten*) *ziczac* in suspended culture. Mar Biol 126:245–251

Mayrand E, Pellerin-Massicotte J, Vincent B (1994) Small scale variability of biochemical indices of growth in *Mya arenaria* (L). J Shellfish Res 13:199–205

Merkel FR, Jamieson SE, Falk K, Mosbech A (2007) The diet of common eiders wintering in Nuuk, southwest Greenland. Polar Biol 30:227–234

Millward DJ, Garlick PJ, James WPT, Nnanyelugo DO, Ryatt JS (1973) Relationship between protein synthesis and RNA content in skeletal muscle. Nature 241:204–205

Millward DJ, Garlick PJ, Nnanyelugo DO, Waterlow JC (1976) The relative importance of muscle protein synthesis and breakdown in the regulation of muscle mass. Biochem J 156:185–188

Moore MN, Allen JI, McVeigh A (2006) Environmental prognostics: an integrated model supporting lysosomal stress responses as predictive biomarkers of animal health status. Mar Environ Res 61:278–304

Norkko J, Thrush SF (2006) Ecophysiology in environmental impact assessment: implications of spatial differences in seasonal variability of bivalve condition. Mar Ecol Prog Ser 326:175–186

Norkko J, Norkko A, Thrush S, Cummings V (2005a) Detecting growth under environmental extremes: spatial and temporal patterns in nucleic acid ratios in two Antarctic bivalves. J Exp Mar Biol Ecol 326:144–156

Norkko J, Pilditch C, Thrush S, Wells G (2005b) Effects of food availability and hypoxia on bivalves: the value of using multiple parameters to measure bivalve condition in environmental studies. Mar Ecol Prog Ser 298:205–218

Norkko J, Hewitt J, Thrush S (2006a) Effects of increased sedimentation on the ecophysiology of two estuarine soft-sediment bivalves, *Austrovenus stutchburyi* and *Paphies australis*. J Exp Mar Biol Ecol 333:12–26

Norkko J, Thrush S, Wells G (2006b) Indicators of short-term growth in bivalves: detecting environmental change across ecological scales. J Exp Mar Biol Ecol 337:38–48

Parsons TR, Maita Y, Lalli CM (1984) A manual of chemical and biological methods for seawater analysis. Pergamon Press, Toronto

Pease AK (1976) Studies of the relationship of RNA/DNA ratios and the rate of protein synthesis to growth in the oyster, *Crassostrea virginica*. Tech Rep Fish Mar Serv Can 622:1–88

Pedersen S (1994) Population parameters of the Iceland scallop (*Chlamys islandica* (Müller)) from West Greenland. J Northwest Atl Fish Sci 16:75–87

Ragnarsson SA, Raffaelli D (1999) Effects of the mussel *Mytilus edulis* L. on the invertebrate fauna of sediments. J Exp Mar Biol Ecol 241:31–43

Riisgård HU, Larsen PS (2005) Water pumping and analysis of flow in burrowing zoobenthos: an overview. Aquat Ecol 39:237–258

Robbins I, Lubet P, Besnard JY (1990) Seasonal variations in the nucleic acid content and RNA-DNA ratio of the gonad of the scallop *Pecten maximus*. Mar Biol 105:191–195

Roddick D, Kenchington E, Grant J, Smith S (1999) Temporal variation in sea scallop (*Placopecten magellanicus*) adductor muscle RNA/DNA ratios in relation to gonosomatic cycles, off Digby, Nova Scotia. J Shellfish Res 18:405–413

Sejr MK, Jensen KT, Rysgaard S (2000) Macrozoobenthic community structure in a high-arctic East Greenland fjord. Polar Biol 23:792–801

Sejr MK, Nielsen T, Rysgaard S, Risgaard-Petersen N, Sturluson M, Blicher M (2007) Fate of pelagic organic carbon and importance of pelagic-benthic coupling in a shallow cove in Disko Bay, West Greenland. Mar Ecol Prog Ser 341:75–88

Sejr MK, Blicher ME, Rysgaard S (2009) Sea ice cover affects inter-annual and geographic variation in growth of the Arctic cockle *Clinocardium ciliatum* (Bivalvia) in Greenland. Mar Ecol Prog Ser 389:149–158

Smith RW, Palmer RM, Houlihan DF (2000) RNA turnover and protein synthesis in fish cells. J Comp Physiol B Biochem Syst Environ Physiol 170:135–144

Storch D, Lannig G, Pörtner HO (2005) Temperature-dependent protein synthesis capacities in Antarctic and temperate (North Sea) fish (Zoarcidae). J Exp Biol 208: 2409–2420

Wright DA, Hetzel EW (1985) Use of RNA-DNA ratios as an indicator of nutritional stress in the American oyster *Crassostrea virginica*. Mar Ecol Prog Ser 25:199–206

Editorial responsibility: John Lawrence, Tampa, Florida, USA

Submitted: December 1, 2010; Accepted: February 11, 2010
Proofs received from author(s): April 23, 2010