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Relating nucleic acid and protein indices to growth in *Mysis relicta*: ration, cycling temperature, and metabolism

Ora E. Johannsson[1,*], Kelly L. Bowen[1], Chris M. Wood[2], Richard W. Smith[2], Christine Chu[3], Lars G. Rudstam[3], Brent Boscarino[3]

[1]Great Lakes Laboratory for Fisheries and Aquatic Sciences, Fisheries and Oceans Canada, 867 Lakeshore Rd., Burlington, Ontario L7R 4A6, Canada

[2]Department of Biology, McMaster University, 1280 Main St. W., Hamilton, Ontario L8S 4K1, Canada

[3]Cornell University Biological Field Station, 900 Shackleton Point Rd., Bridgeport, New York 13100, USA

ABSTRACT: We investigated growth rate, nucleic acid (DNA, RNA) and protein indices and respiration in juvenile (8.5 to 12 mm total body length, 7 to 20 mg wet wt) and young adult (12 to 14 mm, 20 to 30 mg wet wt) *Mysis relicta,* as a function of temperature, body mass and molt stage in order to develop methods to assess condition or growth in the field. Mysids were exposed to either a preferred temperature (6.5°C) and 3 ration levels, or a range of constant and dielly-cycling (DC) temperatures with ad libitum feeding. Mysid growth parameters (specific rates of growth [SGR], respiration [MO_2], and RNA content cell^{-1}) integrated the DC temperature experienced as averaged responses weighted by the time spent at each temperature. MO_2 peaked at 12.7°C on acute temperature exposure from 4.2°C. MO_2 compensation with prolonged temperature exposure occurred at mean diel temperatures ≤8.5°C. Mysids could not survive at 16°C even for 5 h d^{-1}. These results confirm behavioral observations of temperature preferences. RNA concentration in *M. relicta* increased with ration and decreasing temperatures. Protein:DNA ratio, %protein and SGR increased with ration and then plateaued. Protein:DNA ratio, %protein and DNA:weight ratio did not change with temperature with unlimited feeding. Forward, stepwise, multiple regression models for each experiment and the combined data accounted for 31 to 72% of variability in SGR. Our experimental data provide guidance, a preliminary temperature-correction factor for RNA, and benchmarks for use of nucleic acid and protein indices in assessing growth or condition of *M. relicta* in the field.

KEY WORDS: RNA · Monitoring · Respiration · Condition · *Mysis relicta* · *Mysis diluviana*

INTRODUCTION

We are interested in determining whether current ecosystem changes in the Great Lakes (Mills et al. 2003) are impacting the opossum shrimp *Mysis relicta* Lovén, 1862, a key link in the food web between plankton and fish (Johannsson et al. 2003, Owens et al. 2003). Growth is one of the most integrative measures of individual fitness, and therefore is a useful measure of potential effects of ecosystem changes. However, direct measurement of growth rate in the field, even average growth rate, is difficult. Traditionally, cohort analysis has been used (e.g. Morgan 1980). It is often difficult or impossible to apply this technique to *M. relicta* because the mysids can have prolonged periods of reproduction and several overlapping generations (Johannsson 1992). Cohort analysis is also plagued by the unknown influence of size-selective predation and disadvantaged by the long time frame each growth measure represents. Physiological correlates of growth, such as enzyme activity, lipid level and nucleic acid concentration or ratios (Clarke & Walsh 1993, Houlihan et al. 1993, Bergeron 1997) may provide an alternate means of detecting changes. We chose to study nucleic acids because they are closely

*Email: johannssono@dfo-mpo.gc.ca

linked to the production of body mass and appear to change at a time frame of interest for studying species interactions (Foster 1990, Schlechtriem et al. 2008)

Growth rate and protein synthesis rates are often correlated with RNA content, or ratios of RNA:DNA and RNA:protein in both vertebrates and invertebrates (Buckley 1979, Houlihan et al. 1993, Dahlhoff & Menge 1996, Bergeron 1997, Saiz et al. 1998, Wagner et al. 2001). While ribosomes, which are composed largely of ribosomal RNA (rRNA), set the upper capacity for protein synthesis, the actual rate will vary with translational efficiency depending on short-term needs (Millward et al. 1973, Preedy et al. 1988). In order to make comparisons between groups, total RNA must be normalized to a single cell or to body size, usually expressed as total DNA or protein; hence the frequent use of the ratios RNA:DNA and RNA:protein. Metabolically more active cells are larger (Smith et al. 1999), and protein:DNA, a measure of cell size, has also been observed to increase with growth rate (Foster 1990).

RNA concentrations and activities are sensitive to temperature: RNA has a higher translational efficiency at higher temperatures, and consequently, less is required to maintain the same level of growth (Buckley 1982, Foster et al. 1992, Mathers et al. 1993). The relationship between RNA, temperature and growth needs to be examined to define these relationships in each species of interest. RNA:DNA may also change with the size or life stage of an organism, particularly if cellular growth patterns shift between hyperplasia (production of many small cells) and hypertrophy (enlargement of existing cells); e.g. copepod: *Euchaeta norvegica* Boeck, 1872 (Bamstedt & Skjoldal 1980); *Calanus* sp. (Wagner et al. 2001); mysid shrimp: *Mysis relicta* (Nordin 2005). In crustaceans, molting further complicates the interpretation of nucleic acid concentrations and ratios because they may follow distinct patterns through the molt cycle; e.g. lobster: *Homarus* spp. (Juinio et al. 1992), cladoceran: *Daphnia* spp. (Gorokhova & Kyle 2002). Consequently, prior to use as a measure or index, nucleic acid concentrations and growth rate should be quantitatively related in the laboratory for each species, taking into account temperature, size or life stage, and molt stage (in arthropods).

The present study investigates protein and nucleic acid concentrations and ratios in juvenile (8.5 to 12 mm total body length, 7 to 20 mg wet weight [wet wt]) and young adult (12 to 14 mm, 20 to 30 mg wet wt) *Mysis relicta* as a function of growth rate, temperature, body mass and molt stage. These relationships can then be used to assess the condition or growth rates of *M. relicta* in the field. Under ad libitum feeding conditions, size and temperature should be the dominant factors determining growth rate.

We hypothesize that: (1) under ad libitum feeding, nucleic acid:protein indices capture individual variation in specific rates of growth (SGR), and thus, can improve the prediction of SGR beyond that predicted by body size and temperature alone, (2) at a constant growth rate, the decrease in RNA concentration with an increase in environmental temperature would be exponential, indicating that a certain proportion was lost per °C increase in temperature, and (3) protein:DNA increases with temperature as metabolic activity increases, while DNA:weight decreases and % protein remains stable.

Mysis relicta undertakes diel vertical migrations, experiencing a range of temperatures each day; therefore we hypothesize that: (4) *Mysis relicta* cannot maintain a constant respiration rate (accommodation) across the range of normally experienced temperatures and (5) the response of RNA, SGR and mean diel respiration rate to temperature are determined by the mean diel temperature experienced; that is, they are not overly influenced by the high or low temperature extreme experienced during a day.

In the field, SGR will also be determined by food availability and quality, thus we hypothesize that: (6) nucleic acid:protein indices can improve the prediction of SGR when rations are variable, (7) RNA:DNA, RNA:protein, protein:DNA and % protein will increase with increasing SGR while DNA:weight will decrease, and (8) changes in SGR across both temperature and ration can be captured by modeling.

MATERIALS AND METHODS

Environmental conditions. Experimental treatments and conditions were held within the environmental ranges normally experienced by *Mysis relicta. M. relicta* (or *M. diluviana* and its sibling species; Audzijonyte & Väinolä 2005) is a circumpolar, glacial relic inhabiting the deep, colder waters of oligo- and mesotrophic lakes in northern North America and Eurasia. Bottom temperatures in these lakes can range from <1 to 8°C depending on the season and depth of the lake. The population migrates dielly, often between 100 and 200 m at rates of 1 to 2 m min^{-1}, ascending just after dusk into warmer waters in the metalimnion and descending before dawn (Rudstam 2008). Its vertical distribution is governed by temperature, light levels, and likely, food supply (Gal et al. 2004). Although preferred temperatures are in the range of 6 to 8°C for adults and subadults and closer to 12°C for juveniles (Boscarino et al. 2007), individuals often enter the metalimnion at night where they encounter temperatures of 12 to 17°C (Beeton & Bowers 1982). Growth and respiration rates were determined at stable and dielly cycling (DC) temperatures between 4 and 16°C.

Mysis relicta avoids waters with light intensities above 2×10^{-6} mylux or roughly 3.5×10^{-4} lx, the equivalent in Lake Ontario waters (Gal et al. 1999). It is very sensitive to light in the range of 450 to 600 nm, but insensitive to red light. Therefore, the experiments were run under 19 h of darkness and 5 h of red light; all cleaning and feeding of the mysids was done under red light. A double-door system on the experimental room prevented light from entering.

Mysis relicta is an opportunistic feeder consuming prey ranging from detritus and phytoplankton to large, fast-moving zooplankton, other macroinvertebrates and newly hatched fish (Grossnickle 1982, Seale & Binkowski 1988, Nordin et al. 2008). Cyclop-eeze (freeze-dried copepods; Argent Chemical Laboratories) and *Artemia* sp. nauplii were used as the experimental prey.

Temperature effect experiment (TEE): effect of temperature on growth, nucleic acid indices, and oxygen consumption. Stable and DC temperature regimes were established in 114 l plastic tubs housed in a 4°C cold room. A styrofoam platform was fitted inside each tub, about two-thirds of the way up, which suspended 25 glass jars of 250 ml each (Fig. 1). Water filled the tubs to the base of the styrofoam. The temperature of the water was controlled to within ±0.5°C using a time-of-day controller coupled to a temperature probe (Control) suspended in the water near the center of the tub and 2 aquarium heaters (Tronic heater 200W) which warmed the water. The water was circulated using a fountain pump (Beckett M130AUL) to ensure even heating of all jars. The temperature varied only 0.5°C amongst the jars. A StowAway TidbiT temperature logger (Onset), placed in the central jar, tracked temperature for the duration of the experiment.

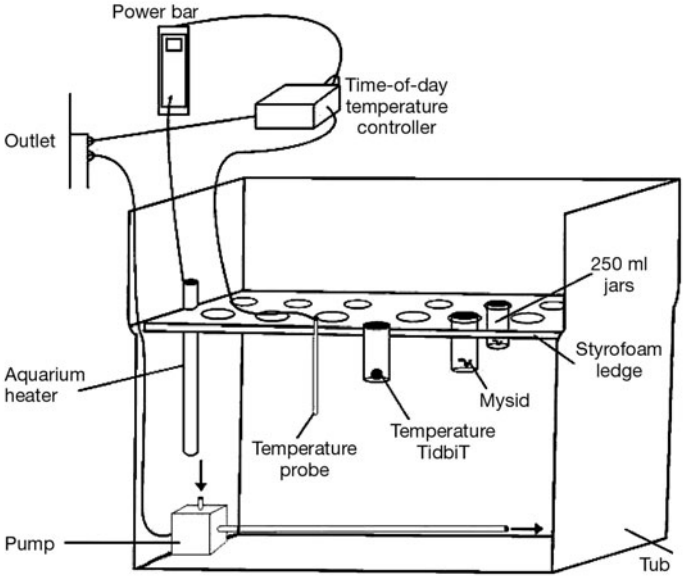

Fig. 1. Schematic of a temperature treatment tub bisected lengthways. The arrows going into and out of the pump indicate the direction of water flow

Four constant (4.2, 8.5, 12.7 and 16°C) and 2 DC (daily mean temperatures: 5.3 and 6.4°C) temperature treatments were established: the DC treatments consisted of 19 h in a 4°C tub and 5 h in an 8 or 12°C tub. Prolonged exposure to 16°C, even for 5 h d^{-1}, proved lethal, so only data from acute effects on respiration at 16.0°C are reported (mean DC temperature: 6.7°C). The jars were moved between tubs by hand at 09:00 and 14:00 h. Temperature in the jars changed exponentially when they were transferred and was within 1°C of the new temperature within 30 min, reaching the final temperature within 1 h. The jars were covered with window screening to prevent mysids from escaping.

Mysids were collected from Skaneateles, a New York Finger Lake, the night of 18 August 2004, and held at 4°C with natural zooplankton. If they were not used for the experiment immediately, they were stored in containers of chlorine-free Lake Ontario water and fed Cyclop-eeze. The jars were regularly cleaned and the water renewed.

One hundred and twenty mysids were weighed twice and placed in the experiment over 19 and 20 August: 24 ind. treatment^{-1}, 1 ind. jar^{-1}. The experiment ran for 22 to 24 d; mortalities were replaced for 5 d; the minimum growth period was 19 d. Nineteen (16%) mysids were replaced, 17 of them in the 12°C treatment. To minimize damage during weighing, mysids were kept in the cold (4°C) and dark in their individual jars until processed. Each mysid was caught with a stiff, spoon-shaped net fitted with large-mesh screening and dried from beneath to prevent damage to the mysid, then weighed in 4°C water. Initial mysid wet weights ranged from 7.71 to 30.06 mg. Each day, the mysids were checked and feed Cyclop-eeze ad libitum. Their water was replaced with aerated, chlorine-free Lake Ontario water of the correct temperature every 3 to 4 d, and exuvia collected, dried and weighed. At the end of the experiment, the mysids were weighed twice to obtain a mean final mass, blotted on a tissue and frozen in individual cryovials in liquid N_2 for nucleic acid and protein analyses. Point-in-the-molt cycle (PIMC) was calculated knowing the time since last molt and inter-molt cycle duration determined for each temperature as the mean of the times between 2 successive molts.

Daily (24 h) rates of oxygen consumption (MO_2) were measured in 2 series, one on mysids caught from neighboring Keuka Lake on 14 July 2004 and held at 4°C for up to 5 to 13 d before acute exposure to the experimental temperature regimes, and a second, on mysids captured from Skaneateles Lake on 18 August

2004, held at 4°C for 2 d and then acclimated to the experimental temperature regimes for 11 to 13 d. Mysids were fed Cyclop-eeze ad libitum except when they were isolated to measure MO_2. Each mysid was held for 24 h in a sealed, gas-tight 100 ml plastic vial at the experimental temperature, submerged in a TEE tub. Five blanks and 10 experimental mysids were run per treatment. The O_2 partial pressure (pO_2) of the water in the vial was measured at the beginning and end of the 24 h period using a Radiometer PHM71 system with Clarke-type microelectrodes (Cameron Instruments) thermostatted to the experimental temperature. Zero and 100% (air-saturated water) calibrations were checked between each measurement. Solubility coefficients from Boutilier et al. (1984) were used to convert observed pO_2 values to O_2 concentrations. Mysid wet weights were determined at the end of the experiment.

Ration effect experiment (REE): effect of ration on growth and nucleic acid indices. Food supply was experimentally varied at a single temperature, 6.5 ± 0.5°C, within the preferred temperature range of this species (Boscarino et al. 2007). A range of prey densities were chosen to provide a low ration (LRT) (20 *Artemia* ind. d^{-1}, 1.1% of body wt of a 20 mg mysid: dry wt/dry wt, assuming a mysid is 20% dry wt), medium ration (MRT) (45 *Artemia* ind. d^{-1}, 2.5% of body weight) and high ration (HRT) (>120 *Artemia* ind. d^{-1}, >6.6% of body weight). *Artemia* sp. mean weight = 0.0022 mg dry wt $ind.^{-1}$ (K. L. Bowen unpubl. data).

Mysids were collected from Lake Ontario on 20 June 2005, fed fresh zooplankton or *Artemia* sp. nauplii and held at 6.5°C. Ninety mysids, 13.4 to 25.3 mg wet wt, were assigned to 3 treatments, 30 ind. $treatment^{-1}$, keeping the size distributions within treatments similar. The experiment was set up over 3 d: 30 ind. d^{-1}, 10 mysids $treatment^{-1}$. The experiment started 27 June. Mortalities were replaced until 13 July, a total of 21 (23%) deaths across all treatments. Mysids were fed and inspected for condition and exuvia daily. Their water was changed every 3 to 4 d. After 5 (LRT, HRT) or 6 (MRT) wk, surviving mysids were weighed, blotted, and frozen in liquid N_2. Their PIMC was calculated as in TEE.

Nucleic acid and total protein determination. RNA and DNA contents of individual mysids were determined spectrophotometrically using the UV dual absorbance (232 nm and 260 nm) method according to the protocols developed by Schmidt & Thannhauser (1945) and modified by Munro & Fleck (1966) and Buckley & Bulow (1987). The method is described in detail in Schlechtriem et al. (2008). These methods have been used repeatedly for fish, crustaceans and mammalian assays and their recovery efficiencies (95.4 to 100% RNA) determined by Mathers et al. (1993) and by one of the co-authors of the present study, Smith et al. (1999).

Protein was determined using the alkaline copper/Folin-Ciocalteu phenol reagent method (Lowry et al. 1951), a method widely used by physiologists studying protein dynamics (e.g. Smith et al. 2000). Standard 5-point concentration curves were created with bovine serum albumin.

In order to provide internal standards to run with these analyses, 10 batches (approximately 0.5 g wet wt each) of fresh, frozen Lake Ontario mysids were processed through the homogenization and PCA wash steps and dissolved in 0.5 mol l^{-1} NaOH. This solution was split into 1.5 ml aliquots and stored at –80°C. Two mysid standards were run with each batch of nucleic acid or protein analyses. A total of 6 batches of experimental samples were analyzed. Replicate variability within and among the standards was low: the coefficient of variation (CV) for total RNA was 0.90%, for total DNA 3.35% and for total protein 1.01%.

Statistical treatment of data. Data are reported as mean ± 2 SE and a significance level of $p \leq 0.05$ was used throughout unless otherwise noted. All statistical analyses were performed in SYSTAT (11.0).

In order to address the possible effect of DC-temperature regimes on SGR and respiration (MO_2), and on RNA concentrations, we predicted values at the DC temperature, using models developed from data from the 3 constant temperatures. Predicted SGRs were calculated by averaging the constant-temperature SGRs weighted according to time (5 and 19 h), at each 1 mg weight increment from 10 to 25 mg using equations derived from the TEE data (see Fig. 2 legend). The observed and predicted data were compared using analysis of covariance (ANCOVA). Mass-specific MO_2 comparisons in non-acclimated individuals were performed in a similar fashion. For RNA, models were developed calculating RNA from DNA or protein, and temperature, using only data from the constant-temperature treatments within TEE. RNA values for DC-temperature treatments were predicted from both their DNA and protein values. The observed and predicted RNA values were compared using chi-squared tests. Significance level in the DC-temperature tests was set at $p \leq 0.025$ as the predicted data had no variability.

Nucleic acid ratios and MO_2 change with body size; therefore, treatment effects were assessed using ANCOVA after ensuring equality of slopes. ANOVA was used to test differences among treatments when covariates were not significant. Tukey's post hoc tests were applied to assess the significance of individual treatment differences. Significant Wet weight × Ratio interaction terms occurred with the ratios of RNA:DNA, RNA:protein, protein:DNA and DNA:weight. Interaction terms were no longer significant if the

ratios were split, e.g. RNA versus DNA instead of RNA: DNA versus wet weight. This allowed the testing of treatment effects within ANCOVA. Linearity and heteroskedascity of relationships were checked visually and from inspection of residuals. SYSTAT indentifies outliers which violate the assumptions of a test. These cases were removed when identified by the program.

Growth rate (G, d^{-1}) and SGR (% d^{-1}) were calculated according to Sutcliffe (1970):

$$G = (\ln(Wt_F) - \ln(Wt_I))/\Delta t \quad (1)$$

$$SGR = 100(e^G - 1) \quad (2)$$

where Wt is the wet weight in mg at the end (subscripted F for 'final') and at the start (subscripted I for 'initial') of the experiment, and Δt is the period of growth in days. Final weight included the weight of exuvia shed during the experiment.

For each experiment, relationships between SGR and potential explanatory variables were sought: temperature, Wt_I and Wt_F, PIMC, the nucleic acid and protein indices (RNA, RNA:Wt_F, RNA:DNA, RNA:protein, protein:DNA, DNA:Wt_F, %protein) and their temperature interaction terms. A Pearson correlation matrix was calculated for all variables including SGR. The explanatory variables that were significantly associated with SGR were examined for significant associations with each other. A regression model predicting SGR was calculated from the explanatory variable with the highest association with SGR plus any other significant independent explanatory variables. A Pearson correlation matrix was then constructed from the residuals and remaining explanatory variables, and the process repeated iteratively until no significant associations existed between the residuals and the remaining explanatory variables. A comprehensive model predicting SGR was then developed by entering the selected explanatory variables into a forward-selection stepwise multiple regression. The residuals of this regression were tested for further associations with the explanatory variables. If significant associations were found, the new variable was entered into the stepwise multiple regression. SGRs predicted from the models were compared with observed SGRs. The adjusted mean R^2 (Adj. R^2), standard error of the estimate (1 SE), number of data points (N) and probability (p) are reported with the models.

RESULTS

Growth

SGR increased with temperature and ration, and declined with mysid Wt_I (Figs. 2 & 3). In TEE, with mysids fed ad libitum, temperature altered relationships between SGR and Wt_I (Fig. 2), SGR being higher in smaller animals and at higher temperatures. No differences in slope were discernable between 4.2 and 6.4°C; however, significant increases were observed at 8.5 and 12.7°C. Nucleic acid-protein indices improved the predictability of SGR beyond that given by temper-

T (°C)	SGR	Adj. R^2	SE	p	N
12.7 (–··–□)	1.27 – 0.049 Wt_I	0.698	0.173	0.001	11
8.5 (–·–○)	0.93 – 0.033 Wt_I	0.264	0.238	0.024	16
6.4 (– –●)	0.50 – 0.014 Wt_I	0.194	0.142	0.030	20
5.3 (——■)	0.39 – 0.007 Wt_I	0.008	0.158	0.292	23
4.2 (·····△)	0.51 – 0.014 Wt_I	0.096	0.179	0.121	17

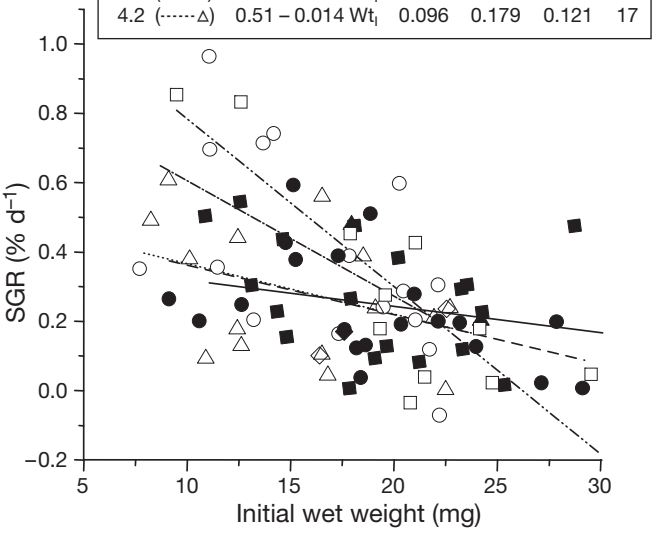

Fig. 2. *Mysis relicta*. Specific growth rate (SGR) as a function of Wt_I (initial wet wt) at a range of mean diel temperatures (temperature effect experiment, TEE). The relationships at 4.2°C and 6.4°C overlap so that only 1 regression line is visible. Mysids were fed ad libitum with Cyclop-eeze

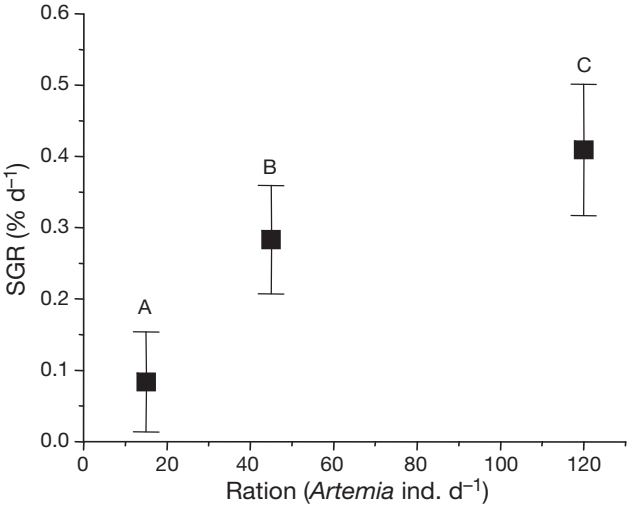

Fig. 3. *Mysis relicta*. Specific growth rates (SGR) at 3 food rations (ration effect experiment, REE; temperature 6.5°C). Means ± 2 SE; dissimilar letters indicate significantly different values

ature and Wt_I alone. When temperature, weight and their interaction term were entered into the forward stepwise multiple regression, 2 points of high leverage were identified. When these points were removed, the equation reduced to:

$$SGR = 0.616 - 0.018Wt_I$$
$$(\text{Adj. } R^2 = 0.196, SE = 0.186, N = 85, p < 0.001) \quad (3)$$

When nucleic acid-protein indices were incorporated in the formation of the model, no outliers or points with high leverage were identified, and RNA:DNA plus RNA:DNA × Temp captured additional variability in SGR:

$$SGR = 0.051 + 0.263\,\text{RNA:DNA} - 0.027\,Wt_I + 0.012\,\text{RNA:DNA} \times \text{Temp}$$
$$(\text{Adj. } R^2 = 0.314, SE = 0.179, N = 87, p < 0.001) \quad (4)$$

where Wt_I is in mg and Temp is temperature in °C.

In REE, as ration increased, SGR increased from 0.08 % d^{-1} (20 *Artemia* ind. d^{-1}) to 0.29 % d^{-1} (45 *Artemia* ind. d^{-1}) and 0.35 % d^{-1} (>120 *Artemia* ind. d^{-1}) (Fig. 3).

Length of the intermolt period, which was determined from mysids which had molted twice during the experiment, decreased with increases in food ration and temperature. In the REE, a period of 25.4 ± 0.6 d was observed at LRT (6.5°C) and was significantly longer than the 22.0 ± 0.6 and 20.8 ± 0.5 d observed at higher rations. TEE ran only 3 wk and double molts rarely occurred. Number of molts per individual increased with temperature from 0.63 at 4.2°C to 1.06–1.08 in the 8.5°C and 12.7°C treatments (Fig. 4), indicating that the intermolt period was reduced at the higher temperatures, reaching a minimum duration near 8.5°C. At the same time, mortality rate increased from 0 % at 4.2°C to 50 % at 12.7°C, reaching 100 % at 16.0°C (Fig. 4).

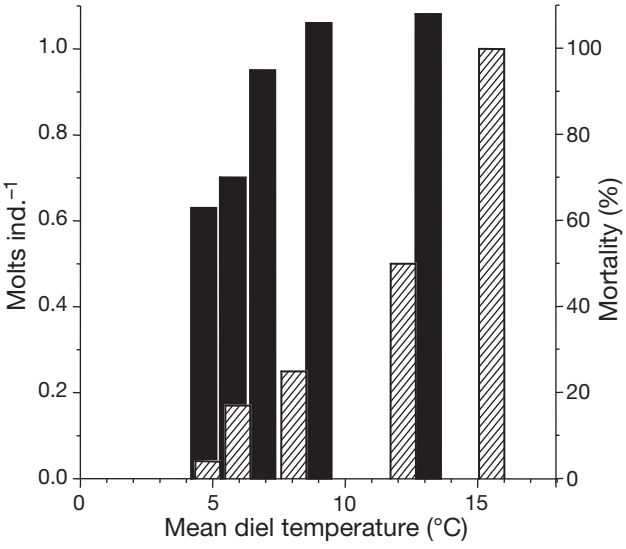

Fig. 4. *Mysis relicta*. Comparison of mortality rates (hatched bars) and molting rates (black bars) over a 3 wk period in mysids inhabiting different diel-temperature (T) regimes, where molting rate = $1.122 - 2.750 \times 0.673^T$ ($r^2 = 0.934$, $\chi^2/df = -0.00567$). Mysids were fed ad libitum with Cyclop-eeze. The 4 to 12°C treatments were from the temperature effect experiment (TEE); the 16°C was from the acute respiration study

Effect of temperature on oxygen consumption

Experimental mysids were held for 5 to 13 d at 4°C prior to testing. Acute exposure to higher temperatures altered their mass-specific MO_2 (Fig. 5A). MO_2 was size-dependent at higher temperatures (≥8.5°C) and increased curvilinearly (Fig. 5B). Q_{10} for 20, 40, and 60 mg wet wt mysids were 2.7, 2.5 and 2.1 respectively, calculated between 4.2 and 12.7°C. MO_2 at 12.7°C and 16.0°C were not significantly different (Fig. 5A,B). Mysids acclimated to DC or constant temperatures for 11 to 13 d expressed respiratory compensation (i.e. maintained constant MO_2 across temperatures) even when exposed to higher temperatures (12.7°C) for 5 h d^{-1}, as long as they had an average daily temperature exposure of <8.5°C. However, the ability to compensate was lost with constant 12.7°C exposure. Mysids could not be acclimated to 16.0°C and individuals exposed to that temperature, even for 5 h d^{-1}, died within 11 d.

Effect of temperature and food ration on indices

In TEE, with ad libitum feeding, ANCOVA analysis indicated that the rate of increase in RNA with DNA was the same irrespective of the temperature regime, in 7 to 30 mg wet wt mysids (Fig. 6A); i.e. the interaction term DNA × Temperature was not significant (common slope = 2.38 ± 0.30). In addition, total RNA was significantly lower at higher temperatures (Fig. 6B). The rate of decline of RNA, with DNA as the covariate (covDNA), with temperature, i.e. the slope of the line through the adjusted means, was −0.93 ± 0.37 µg $°C^{-1}$ (Fig. 6B).

Analysis of protein (covDNA), DNA ($covWt_F$), and %protein indicated that neither cell size, the number of cells per unit of weight, nor %protein (13.5 ± 0.28 %) changed with temperature-related changes in growth rate.

In the REE, Pearson correlation coefficients indicated a positive association between RNA:DNA and protein:DNA ($r \geq 0.67$), but also revealed that RNA:protein and DNA:Wt_F were positively associated with each other ($r \geq 0.50$) and negatively associated with the former ratios ($r > 0.80$).

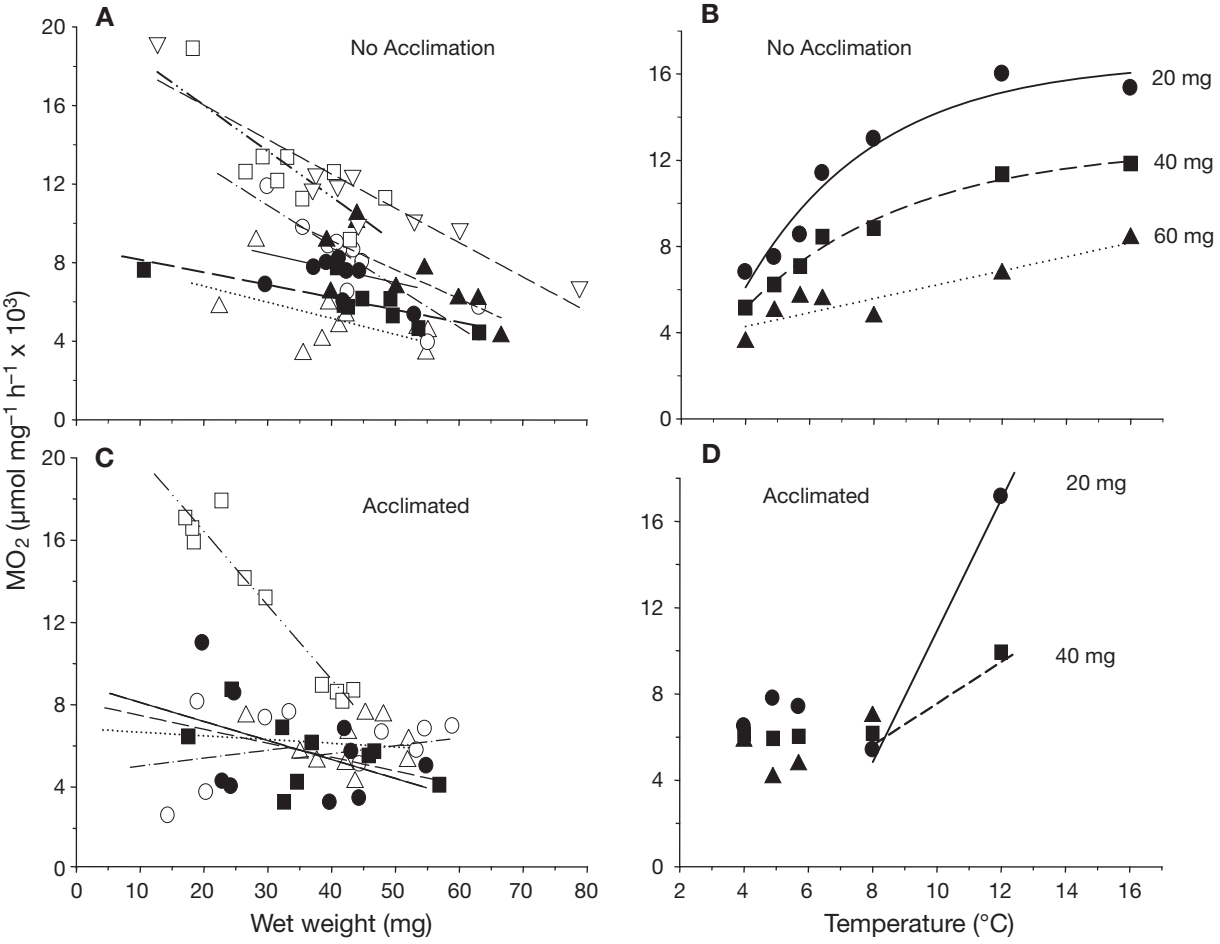

Fig. 5. Mass-specific MO_2 of (A) non-temperature-acclimated and (C) temperature-acclimated mysids with respect to wet weight over a range of mean daily temperature regimes: 16.0 (▽), 12.7 (□), 8.5 (○), 6.7 (▲), 6.4 (●), 5.3 (■) and 4.2 (△) °C; open symbols = constant-temperature treatment, filled symbols = dielly-cycling-temperature treatment. MO_2 in (B) non-temperature-acclimated and (D) temperature-acclimated 20 (●), 40 (■), and 60 (▲) mg wet wt mysids across temperature. Data calculated from relationships in (A) and (C). MO_2 in 20, 40 and 60 mg acclimated mysids experiencing 4.2 to 8.5°C temperatures were not significantly different. Mean MO_2: 6.073 ± 0.602 µmol O_2 mg wet wt^{-1} h^{-1} × 10^3, N = 12. Equations for (B): 20 mg mysid: $MO_2 = 16.596 - 28.154Wt^{0.781}$ (Adj. $R^2 = 0.957$, $\chi^2/df = 0.904$, N = 7), 40 mg mysid: $MO_2 = 12.689 - 16.418Wt^{0.823}$ (Adj. $R^2 = 0.989$, $\chi^2/df = 0.103$, N = 7), 60 mg mysid: $MO_2 = 2.989 + 0.3236Wt$ (Adj. $R^2 = 0.835$, p = 0.004, N = 7)

The relationships within the indices (see Fig. 7), that is RNA relative to DNA, or protein relative to DNA etc., changed with mysid size (Fig. 7A,B,D). The shift occurred at approximately 30 µg DNA, which corresponds with maturation of juveniles into adults. *Mysis relicta* start to show sexual characteristics by 12 mm body length. A 12 mm mysid weighs 20 mg wet wt and contains 30 µg DNA, as determined from the relationship of DNA to wet weight in TEE:

$$DNA = 13.658 + 0.826 \text{Wet wt}$$
$$(\text{Adj. } R^2 = 0.732, SE = 2.3, N = 87, p < 0.001) \quad (5)$$

Protein (covDNA), RNA (covDNA) and protein ($covWt_F$) increased significantly with ration (Figs. 7A–C & 8A,B; adjusted means of protein ($covWt_F$) not shown). Protein ($covWt_F$) is more commonly expressed as %protein. The %protein increased from 11.3 ± 0.20% in LRT to 12.8 ± 0.17% in HRT. However, RNA (cov protein) increased only in the young adults (Fig. 8C). The response of DNA ($covWt_F$) also varied with mysid stage. In juveniles, it was lowest in MRT (Figs. 7D & 8D), while in young adults, it was stable between LRT and MRT, increasing in HRT (Figs. 7D & 8D).

No relationships were detected between RNA or RNA:DNA and PIMC.

Impact of DC-temperature regimes

The following models were developed to predict RNA (µg) from DNA (µg) or protein (mg), and Temp

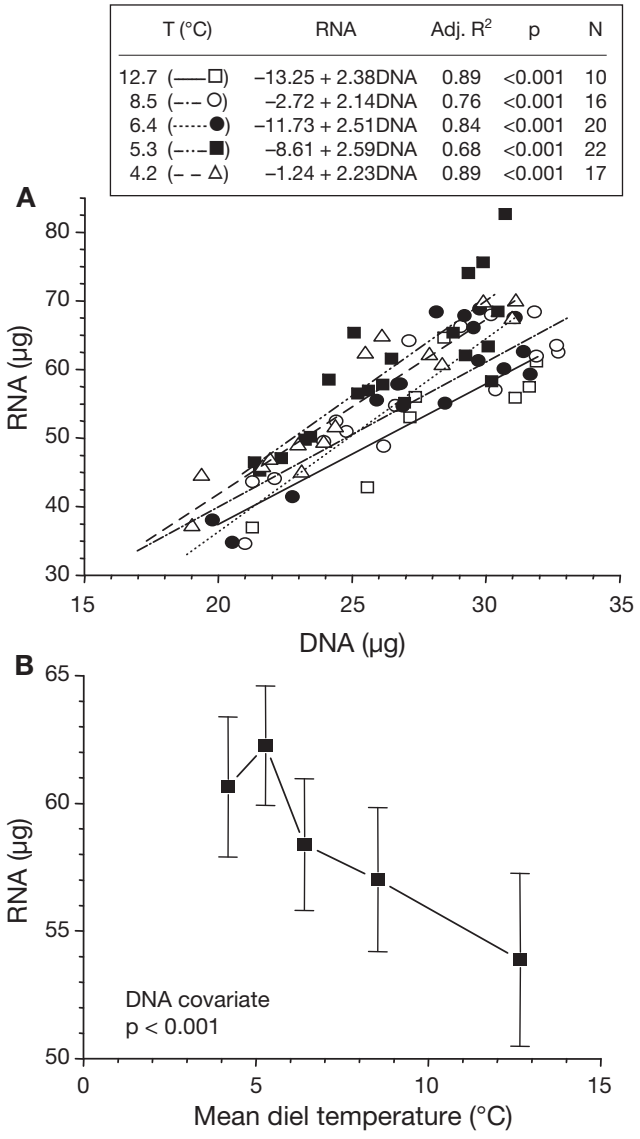

Fig. 6. Relationships from the temperature effect experiment (TEE) between (A) total RNA and total DNA content of mysids at 5 mean diel temperatures, and (B) RNA and temperature (adjusted means ± 2 SE, DNA = covariate). Mysids were fed ad libitum with Cyclop-eeze

(°C), using only data from constant-temperature treatments (TEE):

$$\text{RNA} = 1.948 - 0.794\text{Temp} + 2.227\text{DNA}$$
$$(\text{Adj. } R^2 = 0.836,\ \text{SE} = 4.4,\ p < 0.001,\ N = 44) \quad (6)$$

$$\text{RNA} = 30.975 - 0.921\text{Temp} + 15.038\text{Protein}$$
$$(\text{Adj. } R^2 = 0.815,\ \text{SE} = 4.6,\ p < 0.001,\ N = 44) \quad (7)$$

No significant differences were found between observed and predicted RNA values in either the 5.3°C or 6.4°C DC-temperature treatments, indicating that RNA concentration is a function of mean diel temperature exposure.

The regression relationships between SGR and Wt_I in the DC-temperature treatments were not significantly different from the relationships predicted from the weighted combination of the 2 constant-temperature treatments.

The predicted and observed MO_2 relationships with wet weight in non-acclimated mysids were not significantly different in the DC 5.3°C and 6.4°C treatments. The observed DC 4.2°C to 16.0°C treatment MO_2 rates were higher than those predicted from the weighted mean of the rates at the 2 temperatures. MO_2 in acclimated animals did not differ significantly between 4.2°C and 8.5°C.

Relationships between growth rates and indices

Three equations were developed to predict SGR: 1 for each experiment and 1 for the combined data. Of the potential explanatory variables, Wt_I (mg), RNA (µg), an index based on RNA (RNA:Wt_F [µg mg^{-1}], RNA:protein [µg mg^{-1}], RNA:DNA), RNA:DNA × Temp (°C), and %protein entered the models.

Combined:

$$\text{SGR} = 1.607 + 0.029\ \text{RNA} - 0.352\ \text{RNA:Wt}_F - 0.102\text{Wt}_I + 0.008\ \text{RNA:DNA} \times \text{Temp}$$
$$(\text{Adj. } R^2 = 0.592,\ \text{SE} = 0.133,\ p < 0.001,\ N = 148) \quad (8)$$

REE:

$$\text{SGR} = 3.152 + 0.025\ \text{RNA} - 0.103\text{Wt}_I - 0.058\ \text{RNA:protein} - 0.065\ \%\text{protein}$$
$$(\text{Adj. } R^2 = 0.723,\ \text{SE} = 0.109,\ p < 0.001,\ N = 64) \quad (9)$$

TEE:

$$\text{SGR} = 0.051 + 0.263\ \text{RNA:DNA} - 0.027\ \text{Wt}_I + 0.012\ \text{RNA:DNA} \times \text{Temp}$$
$$(\text{Adj. } R^2 = 0.314,\ \text{SE} = 0.179,\ p < 0.001,\ N = 87) \quad (10)$$

In all cases, the correlations between the observed and predicted SGRs were high and the slopes of the relationships significantly <1. Correlation coefficients of the Combined, REE, and TEE data were 0.749, 0.861 and 0.581, respectively. The slopes of the relationships were 0.60, 0.74 and 0.34, respectively, indicating an underestimation of the true SGRs in all 3 models.

DISCUSSION

Nucleic acid-protein indices can be important tools in assessing the condition and predicting SGR of *Mysis relicta*. Significant relationships between SGR and the responses of these indices were observed as temperature conditions and rations changed. The results can guide the use of these indices in the interpretation of data from field-caught individuals, and provide insight into the growth physiology of *M. relicta*.

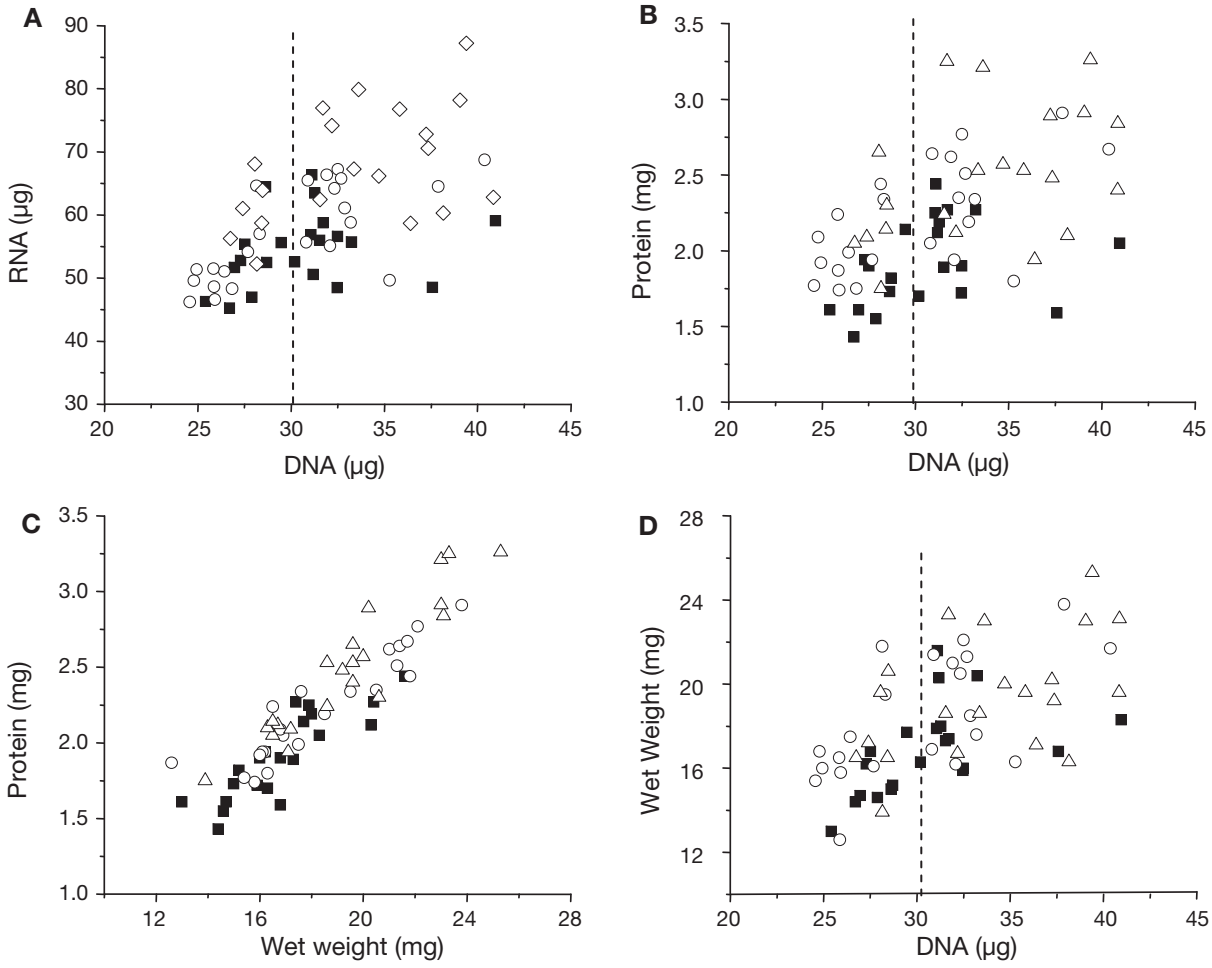

Fig. 7. *Mysis relicta*. Distribution of (A) RNA with respect to DNA, (B) protein with respect to DNA, (C) protein with respect to weight, and (D) weight with respect to DNA at 3 rations: high (HRT, ■), medium (MRT, ○) or low (LRT, △); temperature = 6.5°C. Dashed line is the division between juveniles and young adults at 30 µg DNA

Growth models: inclusion of nucleic acid and protein indices

In accordance with 2 of our hypotheses, nucleic acid-protein indices contributed significantly to the prediction of SGR in models where: (1) only temperature was varied (TEE), (2) only ration was varied (REE) and (3) data from both experiments were combined. The proportion of variance explained by these models, particularly the combined model, was sufficient to confirm the usefulness of these indices in assessing condition and estimating growth rate in *Mysis relicta*: TEE, 31%; REE, 72%; Combined, 60%.

The SGRs predicted from these models were consistently lower than the observed SGRs, although the 2 were highly correlated. This consistent underestimation suggests that other factors not measured in this study may influence the relationship between RNA concentration and growth rate. The relative importance of catabolic and anabolic processes must change as growth rate increases, but this has not been defined. The translational efficiency of RNA may change, perhaps increasing as the availability of the building blocks increases. Although we do not fully understand all the processes at this time, the clear relationship between nucleic acid indices and SGR permits the use of these indices in assessing growth in the field. The predictive SGRs can be 'corrected' to better estimate the observed SGRs by dividing the predicted values by the slope of the relationship between the observed and predicted SGRs.

In the models, RNA occurred either as RNA:DNA or total RNA. The importance of RNA:DNA as a potential indictor of growth has been noted repeatedly, especially in the fish literature (e.g. Houlihan et al. 1993, Bergeron 1997), but also among crustaceans (e.g. Juinio et al. 1992, Saiz et al. 1998, Wagner et al. 1998). Total RNA is less often encountered; however, it increases in a wide variety of ectoderms with increases in rates of protein synthesis (Houlihan 1991) and has

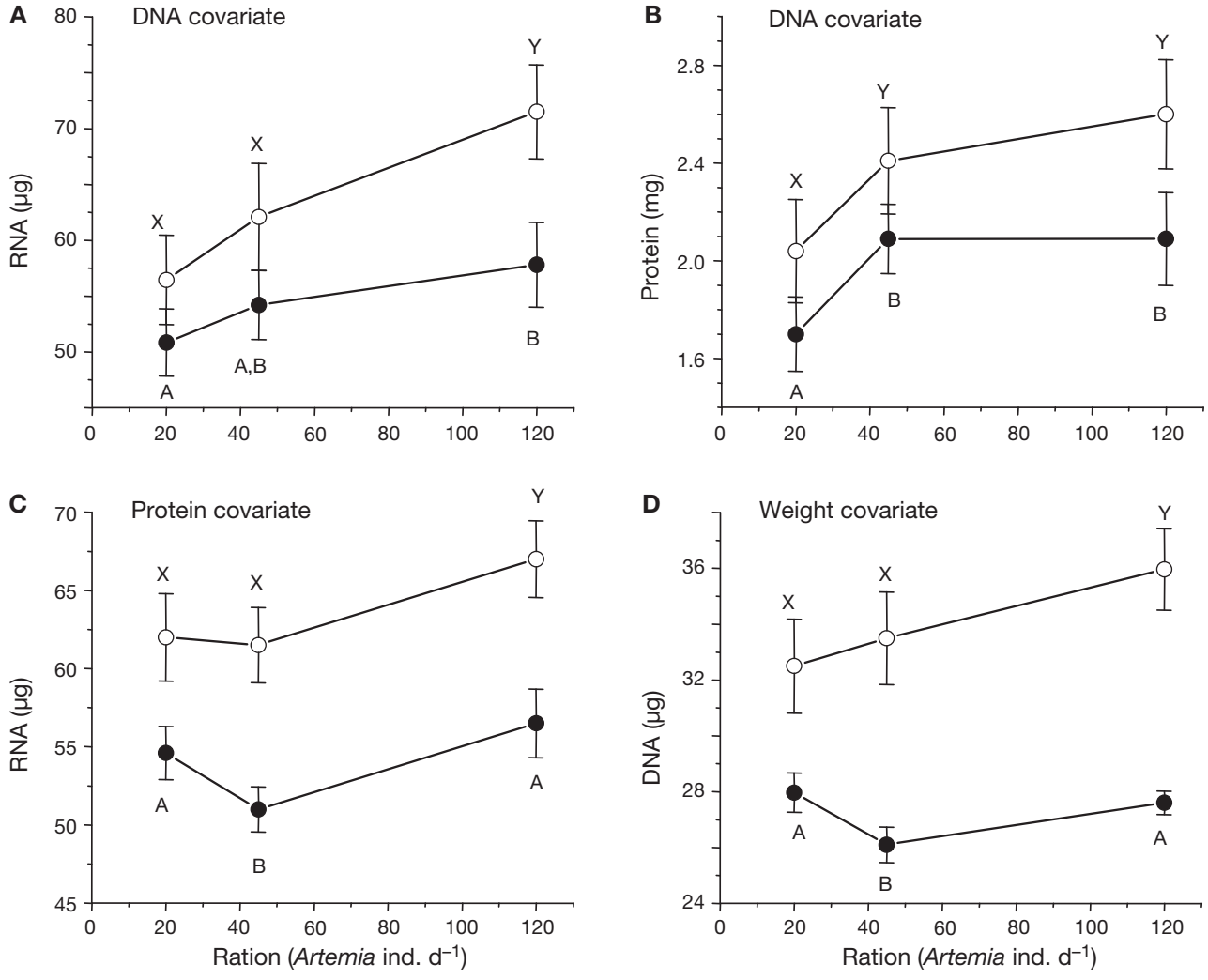

Fig. 8. *Mysis relicta*. Relationship of (A) RNA to DNA, (B) protein to DNA, (C) RNA to protein, and (D) DNA to weight in juveniles (DNA ≤ 30 µg, ●) and young adults (DNA > 30 µg, ○) at 3 ration levels; temperature = 6.5°C. Means ± 2 SE; dissimilar letters indicate significantly different values

also been correlated with growth rate in a variety of organisms (Buckley 1984).

The growth models developed for *Mysis relicta* differ from the growth models developed for other crustaceans. Nucleic acid-based models developed for lobsters, copepods and cladocerans were based on specific life stages or a particular size of individual (Juinio et al. 1992, Saiz et al. 1998, Wagner et al. 2001, Vrede et al. 2002). They successfully related SGR or egg production rate to RNA:DNA changes within that size class. In this way, they dealt with changes in RNA and DNA concentrations and potential growth rate, which occur through development (Peters 1983, Wagner et al. 1998, Nordin 2005), outside the modeling framework. *Mysis relicta* has no series of distinct growth stages to bound the development of growth models, *a priori*, except between adults and juveniles. In addition, in *M. relicta*, cell growth shifts from hyperplasia (increase in same-sized cells) to hypertrophy (enlargement of cells) with age (Nordin 2005), further altering the relationships between nucleic acid ratios and size (e.g. slopes >1, Fig. 7A,B). Wt_I was present in all 3 models, and allowed development of relationships between SGR and nucleic acid-protein indices over a wide range of mysid sizes (7 to 30 mg or 8.5 to 14 mm body length).

Ad libitum SGR and RNA were lower than those observed for Great Lakes' *Mysis relicta* in the field, perhaps reflecting laboratory conditions. Maximum autumn RNA in lakes Ontario and Huron (O. E. Johannsson & K. L. Bowen unpubl. data) were 16 and 37% higher, respectively, than RNA at comparable temperatures in REE-HRT. Growth rates in Lake Ontario calculated over April to October by cohort analysis were 0.035 to 0.029 mm d^{-1} (Johannsson 1992), translating to a range

in SGR of 1.0% d^{-1} (10 mg mysid) to 0.6% d^{-1} (30 mg mysid). In the REE-HRT, SGR was 0.4% d^{-1} (15 to 25 mg mysids), and in TEE (6.4°C), SGR was 0.41 to 0.15% d^{-1} (10 to 30 mg mysids). In future work on these indices, it will be desirable to extend the upper range of SGR, perhaps by including a more varied food supply.

The nucleic acid and protein indices can also be used outside of the models to compare the condition of mysids in different lakes, years or seasons. The present experiments have established benchmarks, at least for the lower ranges of SGR, for juvenile and young adult mysids at commonly encountered diel temperatures (Figs. 6A & 7A–D).

Temperature

Mysis relicta inhabits cold northern lakes where it may experience temperatures between <1°C and 17°C (Beeton & Bowers 1982, Johannsson 1992). Increasing temperatures increase the efficiency of RNA and metabolic processes in general, enabling faster growth rates. We predicted that in response to increasing temperature, RNA would decline exponentially, that protein:DNA would increase while DNA:Wt_F would decrease, that %protein would remain stable, and that these indices could improve the predictability of SGR beyond that due to body size and temperature.

RNA did not decrease exponentially with increasing temperature as would be expected with a proportionate change in efficiency $°C^{-1}$, but rather linearly (Fig. 6A), indicating that the amount of RNA lost $°C^{-1}$ was constant: –0.93 µg $°C^{-1}$. This raises a question which must be addressed before RNA of field-caught animals can be corrected for temperature: How does the rate of RNA loss relate to ration? Can it be predicted from other non-RNA-based indices? Protein: DNA, DNA:weight and %protein are potential candidates since they were not influenced by temperature but did respond to changes in ration in step with SGR (Figs. 7 & 8). In the meantime, they can guide interpretation of changes in RNA in the field. As hypothesized, protein:DNA and %protein increased with increases in SGR, and DNA:Wt_F declined. If these indices indicate that mysids are growing more rapidly, then RNA:DNA should be increasing, and vice versa. Thus, they can flag potential misinterpretation of RNA:DNA due to temperature differences.

Body size and temperature drive differences in SGR (Peters 1983). We hypothesized that the nucleic acid-protein indices could improve the predictability of SGR, under ad libitum feeding, by accounting for some of the individual variability. In TEE, with ad libitum feeding, the adjusted R^2 increased by 60% when the model predicting SGR incorporated nucleic acid-protein indices. However, the model based on Wt_I and temperature relationships did not represent the data well. Two points with high leverage drove the relationship with temperature and when they were removed, the model collapsed to include only Wt_I. Yet temperature obviously impacted SGR (Fig. 2). In the index equation, weight was retained and the temperature effect and individual variability were captured by RNA:DNA and the interaction of RNA:DNA with temperature. This model described the data well: no points exerted undue leverage or had to be removed. The incorporation of temperature in predictions of SGR using RNA:DNA was first recommended by Buckley (1982). Like us, Saiz et al. (1998) found RNA:DNA × Temp improved the prediction of growth in *Calanus finmarchicus.*

Mysis relicta does not live under constant temperature conditions but migrates dielly from the colder hypolimnetic waters into the bottom of the metalimnion or lower surface waters at night. Diel temperature experience ranges from 4 to 17°C (Beeton & Bowers 1982), but is more likely 4 to 12°C for juveniles and 4 to 6–8°C for adults based on their temperature preferences and observed distributions in the field (Gal et al. 2004, Boscarino et al. 2007). We hypothesized that the SGR of a migrating animal could be predicted by averaging the SGRs of mysids at the daytime and nighttime temperatures weighted by the time spent at those temperatures. That is, the benefit of feeding at these higher temperatures was directly proportional to the time spent at each temperature. Both SGR and RNA followed this pattern. Thus, the temperature governing mysid growth and RNA concentrations in the field can be calculated from day length, temperature profiles and knowledge of temperature preference. This enables us to compare mysid data from different seasons or lakes by taking into account their differences in mean diel temperature experience. At the moment, the comparisons can only be directional as exact algorithms for correcting RNA for temperature require further investigation (see 'Discussion – Growth models: inclusion of nucleic acid and protein indices').

Basal metabolic rate indicates the baseline metabolic cost of inhabiting a certain environment. We predicted that there was no inherent metabolic advantage or disadvantage to mysids to undertake diel migrations into warmer surface waters, as judged by their basal metabolic rate (MO_2), and that no compensation would occur upon prolonged exposure to those temperatures. Upon acute exposure to a range of temperatures, respiration rate increased curvilinearly from 4.2 to 12.7°C (Fig. 5). Similar rates of MO_2 have been reported for *Mysis relicta* in the literature (Lasenby & Langford 1972, Foulds & Roff 1976, Sandeman & Lasenby 1980) (Table 1). Respiration rate did not increase between

Table 1. *Mysis relicta*. Literature estimates of mass-specific MO_2 (µmol O_2 mg wet wt^{-1} $h^{-1} \times 10^3$): 40 mg wet weight, 10 mg dry wt. Q_{10} (2.53) of this study for a 40 mg mysid was calculated between 4 (4.2°C) and 12 (12.7°C): the Q_{10} between 4 (4.2°C) and 8 (8.5°C) was 3.85. Animals had access to food except during the experiment in all studies except that of Sandeman & Lasenby (1980) where animals were starved for 3 d prior to the experiment

Source of *M. relicta*	Temperature (°C) 4	5	6	7	8	10	12	15	16	Q_{10}	Comments	Source
Keuka Lake	5.2	6.2[a]	7.1[a]	8.5[a]	8.8	–	11.4	–	11.8	2.53	Acute	Present study
Skaneateles Lake	6.1	5.9[a]	6.0[a]	–	6.2	–	9.9	–	–	–	12 d acclimation	Present study
Char Lake	8.2	–	–	–	10.8	–	–	–	–	1.97	24 h acclimation	Lasenby &
Stony Lake	6.9	–	–	9.3	–	–	–	–	–	2.74	24 h acclimation	Langford (1972)
Kootenay Lake	–	5.7	–	–	–	9.1	–	12.5	–	2.20	Acute, 11 mg l^{-1} O_2	Sandeman &
	–	5.7	–	–	–	8.0	–	11.4	–	2.00	Acute, 8 mg l^{-1} O_2	Lasenby (1980)
Lake Ontario	6.8	–	–	–	–	–	–	–	–	–	Acute	Foulds & Roff (1976)

[a]Fluctuating diel temperature exposure

12.7 and 16°C, suggesting some inability to cope with such high temperatures. Boscarino et al. (2007) found that adult mysids would not go into waters of 16°C unless enticed with prey and then only for brief periods of time. Respiration rates of mysids exposed to a DC of 4.2 to 8.5°C and 4.2 to 12.7°C were similar to those predicted from the weighted means of the rates at the 2 temperature treatments. However, with time (< 11 to 13 d), mysids were capable of compensating their respiration rates for regular diel changes in temperature as long as weighted mean daily temperatures averaged ≤8.5°C. This would include the normal range of migratory temperature experience of mysids through the season in cold water lakes. No compensation occurred if mysids were kept at 12.7°C continuously. This ability to compensate for diel temperature regimes experienced during migration in the field has not been described before and has implications for growth rates. MO_2 compensation enables mysids to commit more consumed energy towards growth at higher temperatures than otherwise expected.

Mysids did not survive at 16°C for 11 d, even at 16°C for 5 h d^{-1}, and mysids with a 4.2 to 16°C DC had higher MO_2 than expected, in spite of the lack of increase in MO_2 between 12.7 and 16.0°C. All evidence indicates that *Mysis relicta* has physiological difficulty coping with a temperature as high as 16°C.

Ration

We predicted that with increased ration and growth rates, cell size: protein (covDNA), RNA content per cell: RNA (covDNA), ribosomal capacity: RNA (covprotein) and %protein would increase while the number of cells per unit body weight: DNA($covWt_F$) would decrease. The indices tracked growth rates but not ration, with 2 exceptions: RNA (covDNA) continued to increase with ration after SGR had stabilized (Figs. 3 & 8A), and RNA (covprotein) remained stable or decreased as SGR increased and then increased at the highest ration (Figs. 3 & 8C). RNA (covDNA) and protein (covDNA) data from fasting *Mysis relicta* followed the predicted patterns, lending support to their use as indicators of 'growth condition' (Schlechtriem et al. 2008).

Increasing RNA concentration is not always an accurate indicator of growth rate (Elser et al. 2003). Acharya et al. (2004) observed that RNA increased although SGR did not in *Daphnia* sp. fed food sufficient in phosphorus but not nitrogen; in this case, phosphorus did not limit ribosome production. Other indices, such as %protein, protein:DNA or DNA:Wt might not change under these circumstances, and can guide interpretation of RNA:DNA trends. On the other hand, if lipids were accumulated more at higher ration levels, this would not be visible in wet weight data as lipids are less dense than water. This would render the growth curve curvilinear as ration increased. Houlihan et al. (1993) used similar arguments to explain the curvilinear shape of SGR in fish when calculated from wet weight. The protein and cell-size indices in our study did not continue to increase between MRT and HRT (Fig. 8B–D), suggesting that nitrogen (or some other nutrient), and not lipid accumulation, was limiting mysid growth in HRT.

Strong correlations between RNA:protein and protein synthesis rates in different tissues within a fish, and RNA:protein and growth in several fish species, led Houlihan et al. (1993) to suggest that it might be a good indicator of growth. In *Mysis relicta*, both RNA and protein content increased with ration; however, protein increased either more rapidly than, or at the same rate as, RNA between LRT and MRT (Fig. 8A,B).

Consequently, no increase in RNA:protein was observed with the increase in SGR. SGR and protein did not increase between MRT and HRT; however, RNA continued to increase with ration resulting in an increase in RNA:protein. Thus RNA:protein was not positively related to SGR in *Mysis relicta*. These dynamics provide a warning: beware of indices where both components change.

CONCLUSIONS

These experiments confirm that nucleic acids and proteins provide information on feeding conditions and growth rates in juvenile and young adult *Mysis relicta,* and that mysids physiologically integrate diel temperature experience in accordance with the time spent at each temperature. The physiological stress suffered at 16°C supports the observed behavioral upper temperature limits of *M. relicta*. Although the SGR models are limited by the smaller-than-observed range of *M. relicta* growth rates, they confirm the utility of developing such models. Presently we can compare field data to our experimental indices and SGRs to evaluate growth rates in the field, and we can employ the indices to answer specific comparative questions, such as: (1) Is the alteration of the zooplankton community by *Bythotrephes longimanus*, an exotic invader to North America, altering mysid growth rates and food availability? (2) Is alteration of the habit and filtering of phytoplankton by another exotic invader, *Dreissena bugensis* (quagga mussels), in mid-depth habitats altering the growth rate of mysids in those regions? (3) Are mysids in shallower depths smaller because they grow more slowly or because larger individuals are consumed or leave? Such questions are not only interesting from the perspective of mysid biology but also for fisheries and ecosystem management.

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