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Effect of Fasting under Different Temperature Conditions on Nucleic Acid Ratios in the Opossum Shrimp *Mysis relicta*: a Calibration Approach

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ABSTRACT. *The opossum shrimp* Mysis relicta *is an important component of the diet of benthivorous and planktivorous fish in the Great Lakes. The invasion of the Great Lakes by exotic invertebrates (*Bythotrephes longimanus, Cercopagis pengoi, Dreissena polymorpha, *and* D. bugensis*) has altered the base and intermediate levels of the foodweb. Thus, information about the condition of* M. relicta *may reveal the extent of indirect effects of these changes on this trophically-important invertebrate. Biochemical indices based on nucleic acid ratios have been shown to be suitable proxies for the growth and condition of aquatic organisms. These indices are affected by multiple factors, such as; food level, temperature, body size, sex/life stage, maturation, and moult stage and need to be calibrated before field data can be interpreted on a quantitative basis. In this study, we investigated the effect of fasting under different temperature conditions on the nucleic acid ratios RNA/DNA, RNA/protein and protein/DNA in* M. relicta. *Juvenile* M. relicta *were exposed to fasting conditions for 11 and 21 d in two controlled laboratory experiments at 3°C and 8°C. Several effects of time and temperature on the condition indices of fasting* M. relicta *were observed; however, we concluded that, of the various metrics tested, only RNA/DNA ratios provide a suitable index of metabolism and condition in fasting animals. RNA concentrations declined in response to fasting on the order of 3–4 d at 8°C and between 4 and 11 d at 3°C. Juvenile* M. relicta *with RNA/DNA ratios < 1.5–1.8 were clearly identified as fasting animals. Field-caught animals having RNA/DNA ratios near these levels are demonstrating clear signs of metabolic stress.*

INDEX WORDS: Mysis relicta, *nucleic acids, condition indices, fasting, calibration.*

INTRODUCTION

The opossum shrimp *Mysis relicta* (Mysidaceae) is a glacial relict native to many deeper North American lakes. In Lake Ontario, it is a major component of both the pelagic and benthic subsystems where it feeds omnivorously on zooplankton, phytoplankton, and detritus (Van Duyn-Henderson and Lasenby 1986, Johannsson *et al.* 2001). It forms a significant diet component of both benthivorous and planktivorous fish (Rand *et al.* 1995). Thus, *Mysis* occupies a pivotal position in Lake Ontario's food web in that it serves as a link between lower

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and higher trophic levels (Grossnickle 1982, Johannsson *et al.* 2003, Mills *et al.* 2003).

The Laurentian Great Lakes have been invaded by a number of exotic invertebrates. By the late 1990s, four Ponto-Caspian species (*Bythotrephes longimanus*, *Cercopagis pengoi*, *Dreissena polymorpha,* and *D. bugensis*) became integral components of Great Lakes food-webs. The full ecological impacts of these invasive species are only partly understood, but it is already clear that food-web relationships have changed in several significant ways (Johannsson *et al.* 2000, Benoît *et al.* 2002, Laxson *et al.* 2003). Consequently, information about the condition and growth of *Mysis* is required to reveal whether the disruptive impacts of these aquatic invaders on the food web exert negative impacts on mysids.

Biochemical indices based on nucleic acids are widely used in assessing growth and condition of fish, fish larvae, and aquatic invertebrates (e.g., Bulow 1987, Mathers *et al.* 1994, Bergeron 1997, Wagner *et al.* 1998, Buckley *et al.* 1999, Vrede *et al.* 2002). While the amount of DNA is accepted as being constant within a cell, total RNA content, 80%–85% of which is ribosomal RNA ($_{(r)}$RNA) (Millward *et al.* 1973, Westerman and Holt 1988) can vary. The RNA/DNA ratio can be used as a proxy for metabolic/synthetic activity. For example, the RNA/DNA ratio was related to somatic growth in herring (*Clupea harengus*) (Folkvord *et al.* 1996) and also showed a decline in starved herring larvae (Clemmesen 1994). A similar result was found in brown trout (*Salmo trutta*) fed a reduced dietary ration (Grant 1996). Similarly, RNA/DNA ratios increased in fed pollack (*Theragra chalcogramma*) compared with starved animals (Canino 1994).

Total RNA content, expressed relative to total protein (μg RNA · mg protein^{-1}), is considered an index of ribosome number which, in turn, represents the absolute upper limit or capacity for protein synthesis (Millward *et al.* 1973). As a result, changes in RNA content are often reflected by a change in protein synthesis rate (e.g., Houlihan 1991, Smith *et al.* 1996).

The protein/DNA ratio is an index of cell size and can also be used as a measure of cellular metabolic activity since there is evidence that larger cells tend to be metabolically more active than smaller cells (Schmidt and Schiber 1995). Mathers *et al.* (1994) have even suggested that protein/DNA might be a better index of growth than RNA/DNA in wild herring.

Condition indices based on these various nucleic acid ratios have been recommended for use in field studies (Bergeron 1997, Buckley *et al.* 1999, Menge *et al.* 2002), and a similar approach could also yield useful information on the growth and condition of field-caught *Mysis*. However, since such indices can be affected by temperature, body size, maturation, and, in the case of crustaceans, moult stage, it is advantageous for these indices to be calibrated before field data can be interpreted on a quantitative basis. Therefore, our goal was to quantify changes in nucleic acid ratios in fasting juvenile *Mysis* and thereby develop a quantitative index of "stress," which might be useful for evaluating the physiological condition of field-caught *Mysis*. In order to determine the time required to induce the first significant response in nucleic acid ratios and to establish index values indicative of "stress," juvenile *Mysis* were exposed to fasting conditions for 1 to 4, 11, and 21 d at two different temperatures (3°C and 8°C). Fasting *Mysis* were compared to control animals which were fed *Artemia* nauplii during the experimental period.

MATERIALS AND METHODS

Mysis Fasting Protocol

Juvenile *Mysis,*10 to 25 mg wet weight, were collected from the offshore regions of Lake Ontario on two occasions: once on 6 April 2004 (Station 65, 43°35′48″N; 78°48′9″ E, 160-m depth); and once on 6 April 2005 (Station 403, 43°35′38″ N; 78°13′37″ E, 184-m depth). Samples were collected at night using 1-m^2 vertical net tows, taken from 2 m above the substrate to the surface. The nets were fitted with windowless cod ends to keep the mysids in water and to prevent temperature shock. The animals were held in 4°C water during transport to the laboratory at the Canada Centre for Inland Waters (CCIW), Burlington, Ontario. The *Mysis* were kept in darkness in a temperature-controlled room (3°C ± 0.3°C) which approximated natural conditions as closely as possible.

For the first experiment (April 2004), centrifuge vials (50 mL) served as the experimental containers. Windows (1.3-cm diam.) were cut at each end and covered with nylon screen (2-mm nylon mesh) to allow water flow through the container. A total of 180 *Mysis* were placed in separate vials to prevent cannibalism. The vials were equally distributed among six 50-L aquaria (30 vials/aquarium) and placed horizontally in submerged racks, in a constant current of aerated water. The water temperature in tanks 1–3 was the same as the environ-

mental chamber temperature (3°C ± 0.5°C). Water temperature in tanks 4–6 was kept at 8°C ± 0.5°C by means of aquarium heaters. Water in the tanks was changed weekly with aerated (for 1 week to remove chlorine) and cooled (to the experimental temperature) municipal drinking water originating from Lake Ontario.

During the second fasting experiment (April 2005), centrifuge vials were replaced by glass Mason® jars (250 mL) as experimental containers to improve the control of the experimental animals. A single *Mysis* was placed in each of 40 jars. Half of the jars were placed in each of two temperature-controlled rooms, one at 3°C and the other at 8°C. As in the first experiment, water was replaced weekly with pre-conditioned municipal drinking water.

Only larger juvenile *Mysis* (9–12 mm body length) were included in the experiments. The fasting protocol consisted of sampling a random set of animals at the beginning of the experiments (time zero) and then at regular time intervals thereafter. During the first experiment, 12 fasting animals were sampled every day until Day 4 followed by a final sampling on Day 11. There was a single additional sampling (Day 21) in the second experiment. This experiment was extended to 3 weeks since the results of the first experiment suggested that a state of complete starvation had not been reached in 11 d. Treatment size was increased to 15 animals. No molting *Mysis* were observed during the experiments. However, it cannot be excluded that exuvia were ingested by the fasting animals. Upon sampling, each animal was weighed individually on a Mettler AE100 balance and immediately flash-frozen in liquid nitrogen to avoid any degradation of nucleic acids. All samples were stored at –85°C until nucleic acid and protein analysis could be carried out.

During the second experiment, two sets of juvenile *Mysis* (n = 15) were fed *ad libitum* newly hatched *Artemia* nauplii. Both control groups were kept under the same experimental conditions as the fasting animals.

RNA-DNA Extraction

All samples were initially homogenized in 0.2 M perchloric acid (PCA) with an IKA Ultra-Turrax T8 homogenizer and Teflon pestle. Samples were kept on ice to avoid degradation of nucleic acids. The PCA soluble fraction containing free amino acids and nucleotides was separated from insoluble components, such as protein and nucleic acids, by centrifugation (10,000×g, 4°C, 8 min) and discarded. The insoluble homogenate was washed twice (as described above) with fresh 0.2 M PCA to ensure a complete removal of soluble compounds. The insoluble homogenates were then dissolved in 0.2 M NaOH. This was assisted by incubation for 1 h at 37°C. The NaOH solutions were used to determine the concentration of RNA, DNA, and protein in all individual animals. Total protein concentrations were determined according to Lowry *et al.* (1951) using bovine serum albumin (BSA, 50–250 μg · mL^{-1}) as the protein standard (Houlihan *et al.*1986, 1988). RNA was extracted from the NaOH solutions (Mathers *et al.* 1993) by precipitation of proteins and DNA using 20% PCA. RNA concentrations were determined in the supernatant after centrifugation (10,000×g, 4°C, 8 min) by a modification of the Schmidt-Thanhauser dual absorbance (260 and 232 nm) method (Munro and Fleck 1966). The total RNA concentrations were calculated as described by Ashford and Pain (1986) and were expressed as μg RNA per mg wet weight. DNA was extracted from the precipitated material obtained during the RNA analysis. The pellet was washed twice with 2% PCA, resuspended in 0.6 N PCA and incubated for 30 min at 70°C to release the DNA into solution (Mathers *et al.* 1993). DNA concentrations were determined again by the dual absorbance technique (Munro and Fleck 1966).

Statistical Analysis

Our goal was to quantify the effects of temperature and time of fasting on nucleic acid ratios in juvenile mysids. ANCOVAs were used to test treatment effects on the nucleic acid ratios with a measure of body size as the covariate because the ratios change as the animals grow. Linearity was checked before proceeding with any analysis and natural log transformations were applied, if necessary, to achieve homoscedascity. The similarity of slopes between treatments was tested using general linear models (significance of interaction terms) and the similarity of intercepts, using ANCOVA: all statistics were performed in SYSTAT (version 11.0).

When the relationships between the nucleic acid ratios, body size (wet weight), and treatments (time and temperature) were examined, there were significant interaction terms between the treatments and body size indicating that the slopes of the relationships differed amongst treatments. Further analyses

of the ratios using ANCOVA techniques were therefore not possible. However, if we tested the numerator of the ratio using the denominator as the covariate instead of wet weight, the interaction terms were no longer significant, with one exception. That is, we tested for the response of RNA to the treatments, with DNA as the covariate, instead of RNA/DNA with wet weight as the covariate. This analysis is clearer because the ratios already include a measure of body size and, if both components are changing simultaneously, as in RNA/protein, it is difficult to elucidate what is happening. Where significant interaction terms still arose, we analyzed the temperature treatments separately to evaluate the effect of fasting.

The slopes of all RNA vs DNA treatments from both experiments and the Day 0 RNA vs DNA data were not significantly different from each other enabling us to incorporate the data from both experiments in the same ANCOVA analysis. From this analysis, the decline in RNA with fasting could be compared for both temperatures. "A posteriori" least significant difference tests were used to test for significant differences between sampling dates and between temperatures.

We also present the relationships between the actual ratios and body weight for the two temperature treatments and time zero. From the presented relationships, comparisons can be made to similar ratios measured in field-caught animals. Therefore, the ratios become the working indices.

RESULTS

All nucleic acid condition indices decreased in response to fasting in both temperature treatments. Total RNA (DNA as covariate) started to decline at an earlier date and fell further at 8°C than at 3°C (Fig. 1A). Total RNA content in animals fasting at 3°C was stable for at least 4 d but decreased significantly by Day 11 ($p = 0.010$). In animals kept at 8°C, a clear decrease in RNA content was observed by Day 4 ($p < 0.001$). Animals fasting for 21 d at 8°C had a significantly lower RNA content than those at 3°C ($p < 0.001$). *Mysis* fed *Artemia* nauplii at 3°C and 8°C for 21 d and those measured at Day 0 all had comparable RNA contents which were significantly higher than in fasting animals (Fig. 1A).

The response of total RNA (total protein as covariate) of juvenile *Mysis* to fasting differed with temperature and time (Fig. 1B). A clear decline of total RNA could be observed after 4 d of fasting at 8°C ($p < 0.01$), while no effect was observed in animals kept for 11 d at 3°C (Fig. 1B). Animals starved for 21 d showed a significant decline in RNA at both temperatures ($p < 0.001$), and declines in RNA concentrations differed significantly between the two temperatures ($p < 0.05$). The RNA contents of the two *Artemia*-fed control groups were comparable and lower than the group at time 0 (Fig. 1B).

Analysis of covariance (on ln of total protein with ln of DNA as a covariate) indicated that both temperature ($p < 0.001$) and fasting time ($p < 0.05$) had a significant effect on total protein concentration (Fig. 1C). A decline in protein occurred between Days 3 and 4 at 8°C ($p < 0.001$) in Experiment 1 and was also observed in Experiment 2 after 21 d of fasting ($p < 0.001$). The protein content of *Mysis* kept at 3°C had also declined by Day 21 but was still higher in comparison to the animals kept at 8°C. The protein contents of the two control groups which were fed *Artemia* nauplii were comparable and had not declined by the end of the experiment.

After assessing the timing and effect of fasting on nucleic acid and protein concentrations, we looked at the ratios and how they responded to body size and to fasting and temperature. Generally, the ratios could not be analyzed, per se, because the slopes of the regression lines changed with time. However, the significant trends in RNA or protein described above also clearly indicate that the ratios changed significantly with fasting. RNA/DNA ratios increased linearly with wet weight (Figs. 2 and 3, Table 1). Comparable ratios (1.8–2.4 for 10–25 mg wet weight animals) were determined for field-caught *Mysis* sampled in Spring 2004 and 2005. Fasting had a clear effect on the RNA/DNA ratios which were highly influenced by the length of the experiment and water temperature. In both experiments, animals fasting at 8°C showed lower ratios compared to their counterparts kept at 3°C. The lowest RNA/DNA ratios (~1.5) where observed in *Mysis* after 21 d of fasting at 8°C. By this time, the slopes of the regression lines fitted to the RNA/DNA ratios flattened with increasing water temperature and showed decreasing r^2 values.

RNA/protein ratios declined linearly with wet weight (Figs. 2 and 3, Table 1). Fasting juvenile *Mysis* for 11 d had no effect on the RNA/protein ratio. Decreases were only observed at 21 d. The RNA/protein ratios had clearly declined, but no effect of temperature could be observed (Table 1).

Like RNA/DNA ratios, protein/DNA ratios in juvenile *Mysis* increased linearly with wet weight

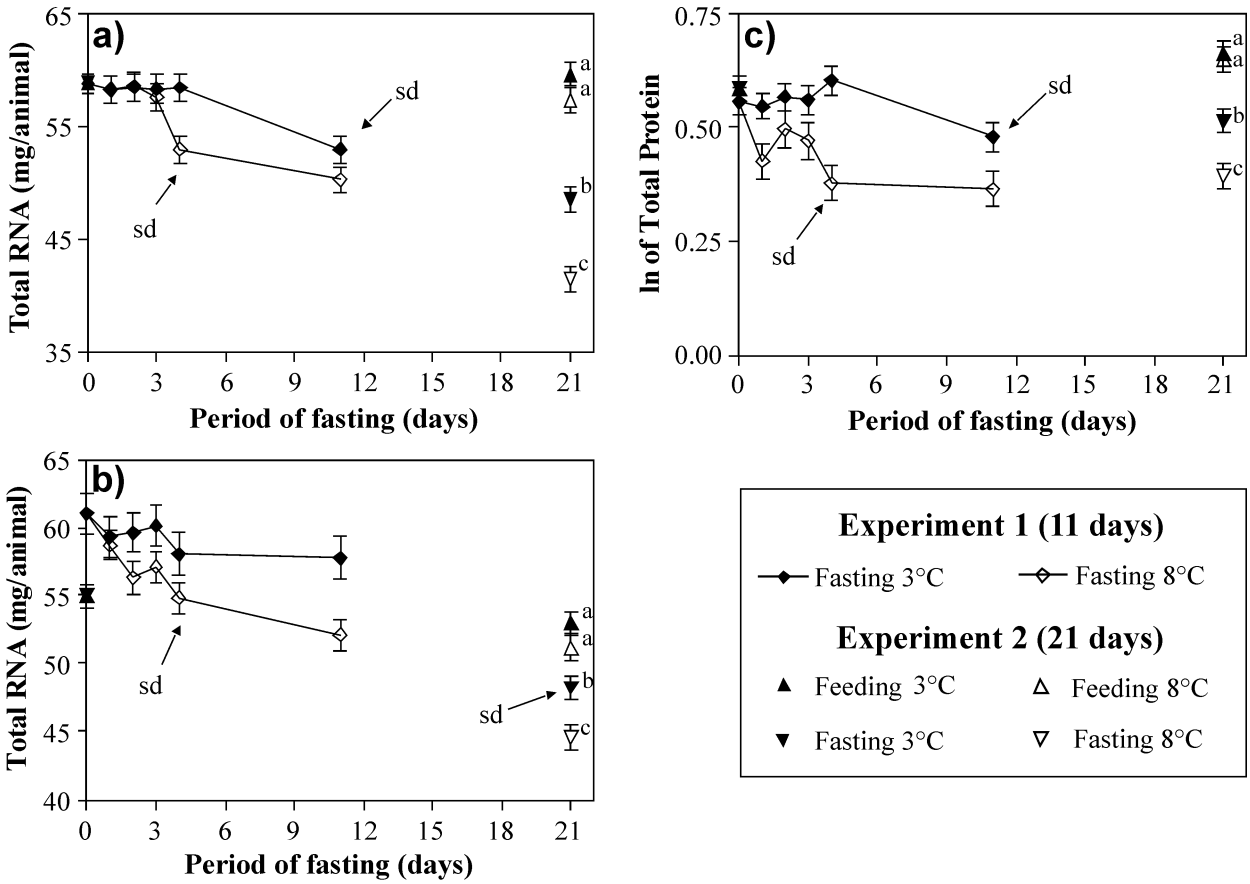

FIG. 1. Changes in a) total RNA with respect to a common DNA concentration (covariate), b) total RNA with respect to total protein (covariate), and c) ln of total protein with respect to ln of DNA (covariate) with fasting in* Mysis relicta *taken from Lake Ontario in April 2004 (Experiment 1; 11 days) and 2005 (Experiment 2; 21 days). Adjusted means ±1 S.E.; 'sd' indicates the day of the first significant decline in RNA (or protein) during fasting at 3°C and 8°C. Individual measurements (Exp. 2) having different superscript letters are significantly different.

(Figs. 2 and 3, Table 1). Similar protein/DNA ratios, increasing from 0.05 to 0.09, could be observed in field-caught animals sampled in 2004 and 2005. *Mysis* fasting for 11 d showed lower ratios (0.04 to 0.08); however, no temperature effect could be observed. During the second experiment, a similar decrease in protein/DNA ratios occurred, although, only animals fasting at 8°C were affected by temperature.

DISCUSSION

Relationship Between Nucleic Acid Ratios and Weight

In juvenile *Mysis*, both RNA/DNA and protein/DNA ratios increased with body size. The presence of these positive relationships indicates that average cell size increased as the animals grew, suggesting there is an increase in hypertrophic growth (growth by cell size) with age. Similar observations have been made for *Mysis* from other lakes in Ontario (Nordin 2005), the American lobster (*Homarus americanus*; Juinio *et al.* 1992), and for herring larvae (*Clupea harengus*; Mathers *et al.* 1994).

The decline in RNA concentration relative to total protein with increases in mysid body mass indicates that smaller mysids have a higher, per unit biomass, capacity for protein synthesis. This observation is common in the animal kingdom where growth rates are usually more rapid in younger animals, presumably because of their overall higher

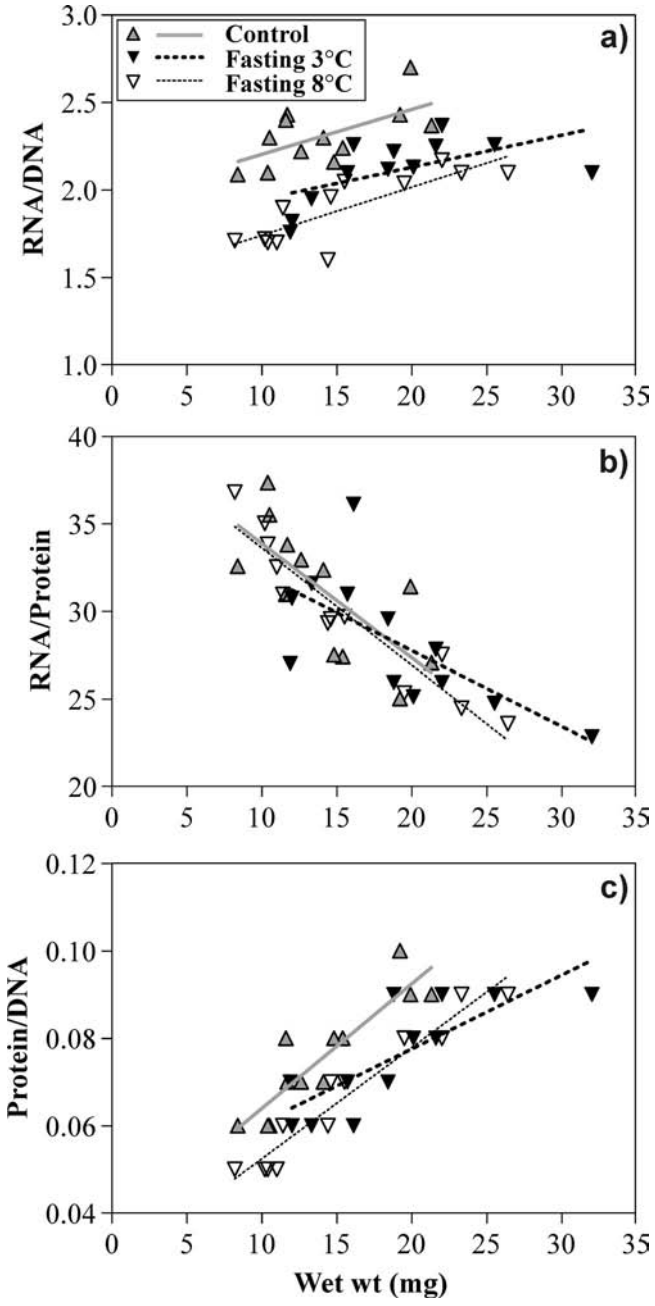

FIG. 2. Relationship between nucleic acid ratios [a) RNA/DNA, b) RNA/protein, c) protein/DNA] and wet weight in **Mysis relicta** *taken from Lake Ontario and after fasting for 11 d at 3°C and 8°C.*

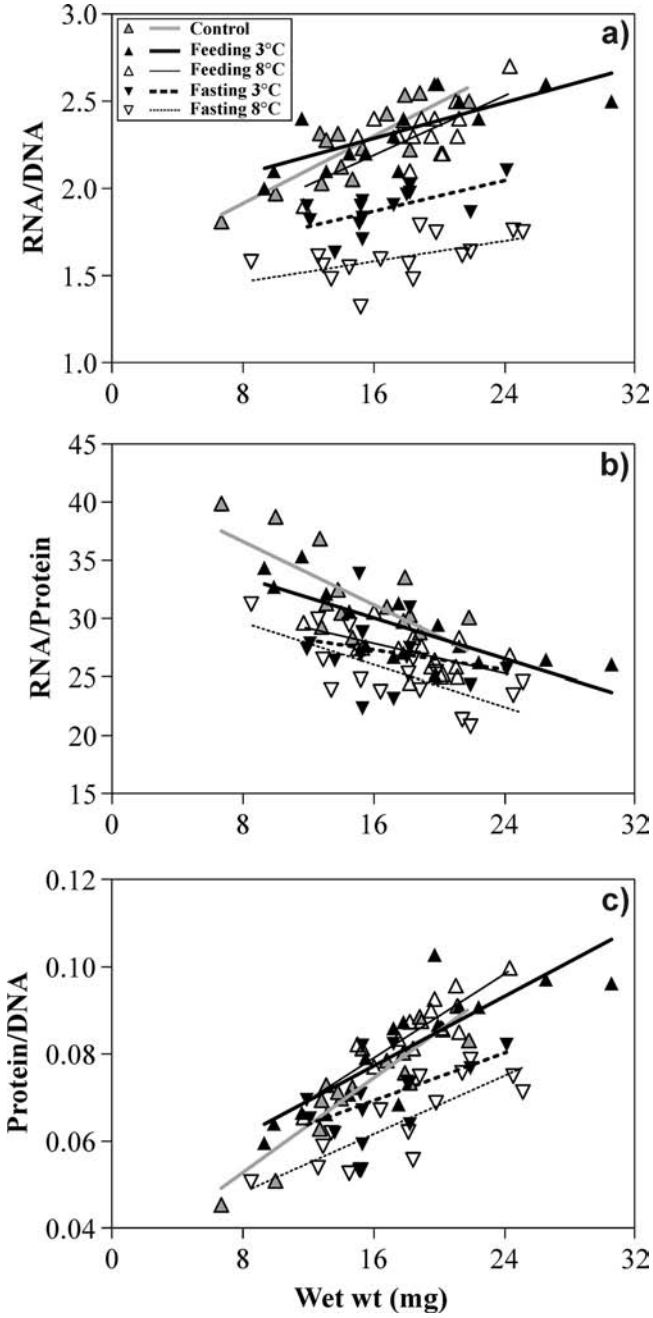

FIG. 3. Relationship between nucleic acid ratios [a) RNA/DNA, b) RNA/protein, c) protein/DNA] and wet weight in **Mysis relicta** *taken from Lake Ontario and after fasting or feeding for 21 d at 3°C and 8°C.*

relative protein synthesis rates. We can hypothesize that a shift toward a hypertrophic growth pattern is one way animals can compensate for a lower metabolic capacity with increasing size: a given biomass comprised of larger cells demands less protein to be synthesized and maintained than the same biomass of more numerous, smaller cells.

TABLE 1. Regression equations and statistical parameters for the relationship between wet weight and the nucleic acid based indices RNA/DNA, RNA/protein, and protein/DNA in field-caught* Mysis relicta *(Control) and animals removed from the lake at the same time but exposed to fasting or feeding conditions for 11 d (Experiment 1) or 21 d (Experiment 2) at 3°C or 8°C.

Dependent variable	Treatment	n	Regression equation	r^2	p
Experiment 1 (11 days)					
RNA/DNA	Control	12	y = 0.02565x + 1.948	0.38	< 0.05
	Fasting 3°C	12	y = 0.01845x + 1.762	0.34	< 0.05
	Fasting 8°C	12	y = 0.02757x + 1.466	0.67	< 0.01
RNA/Protein	Control	12	y = –0.6523x + 40.41	0.52	< 0.01
	Fasting 3°C	12	y = –0.4323x + 36.41	0.47	< 0.01
	Fasting 8°C	12	y = –0.6705x + 40.35	0.89	< 0.0001
Protein/DNA	Control	12	y = 0.002855x + 0.035	0.81	< 0.0001
	Fasting 3°C	12	y = 0.001692x + 0.044	0.64	< 0.01
	Fasting 8°C	12	y = 0.002545x + 0.027	0.93	< 0.0001
Experiment 2 (21 days)					
RNA/DNA	Control	15	y = 0.04836x + 1.526	0.70	< 0.0001
	Feeding 3°C	15	y = 0.02568x + 1.877	0.54	< 0.01
	Feeding 8°C	15	y = 0.04271x + 1.508	0.50	< 0.01
	Fasting 3°C	14	y = 0.02204x + 1.518	0.38	< 0.05
	Fasting 8°C	15	y = 0.01477x + 1.346	0.31	< 0.05
RNA/Protein	Control	15	y = –0.6813x + 42.08	0.46	< 0.01
	Feeding 3°C	15	y = –0.4363x + 37.02	0.62	< 0.001
	Feeding 8°C	15	y = –0.3216x + 33.01	0.30	< 0.05
	Fasting 3°C	14	y = –0.2121x + 30.68	0.06	**0.401**
	Fasting 8°C	15	y = –0.4521x + 33.29	0.51	< 0.01
Protein/DNA	Control	15	y = 0.002716x + 0.031	0.80	< 0.0001
	Feeding 3°C	15	y = 0.002x + 0.045	0.73	< 0.0001
	Feeding 8°C	15	y = 0.002x + 0.041	0.8	< 0.0001
	Fasting 3°C	14	y = 0.001372x + 0.047	0.27	**0.054**
	Fasting 8°C	15	y = 0.001676x + 0.035	0.66	< 0.001

Effect of Fasting on Nucleic Acid Ratios

The final nucleic acid ratios of juvenile *Mysis* fed *Artemia* nauplii were comparable with the ratios from the control animals sampled at the start of the experiment. Thus, the declines in nucleic acid concentrations and ratios in fasting mysids can not be attributed to stressful experimental conditions, but rather were the result of the temperature and fasting treatments. Metabolic effects of fasting are dependent on multiple variables such as the type and availability of metabolic stores and their routes of mobilization (Navarro and Gutierrez 1995). In contrast to a wide variety of marine crustaceans, such as copepods which use wax esters as the principal storage of lipid, freshwater crustaceans, such as *Mysis*, largely accumulate triglycerides (Cavaletto and Gardner 1999). Lipids are the primary energy source of crustaceans (Chang and O'Connor 1983). However, there is evidence that animals which encounter fasting conditions can also catabolize nitrogenous substances (protein) to meet their needs (Claybrook 1983). This is supported by our observation that fasting mysids showed a small but significant decline in their protein content which was reflected in decreasing protein/DNA ratios.

Effect of Temperature and Time on Nucleic Acid Ratios in Fasting *Mysis*

To fully exploit changes in indices, such as those investigated here, one needs to determine the time

frame over which these changes occur. The metabolism of an organism responds to ambient conditions. Processes, such as respiration rate or translational efficiency of RNA, may respond almost immediately while cellular structures take longer to change. It is also clear that the metabolism of an organism responds to ambient conditions. One benefit of the present study is that it provides an estimate of how nucleic acids and protein concentrations in fasting *Mysis* change over time and with respect to temperature. Generally we observed a shorter response time under the higher temperature treatment. Few estimates of response time have been reported in the literature, and the need to rectify this has been recognized (Menge *et al.* 2002). In fasted postlarval Pacific white shrimp (*Penaeus vannamei*) held at 27°C, for example, RNA/DNA ratios declined after the first day of fasting. Similarly, in the American lobster (*Homarus americanus*) RNA/DNA ratios responded to fasting within 2 to 3 d to food deprivation (15, 18 and 21°C) and continued to decline, albeit slowly after the first 10 d, until the end of the experiment on day 20 (Juinio *et al.* 1992).

Temperature influenced the response of juvenile mysids to fasting. When lakes are thermally stratified, *Mysis* normally experience temperatures from 4°C to 15°C during diel vertical migration (Rudstam *et al.* 1999), with a mean diel temperature exposure of ~5 to 6°C in Lake Ontario. Strong winds mix the water to beyond the 100-m depth in winter. At these times, mysids can inhabit temperatures well below 4°C, sometimes as low as < 1°C (Johannsson 1992). As would be expected, temperature has strong effects on mysid metabolism (Berrill and Lasenby 1983, Chipps 1998), and the same is true for nucleic acids.

The RNA/DNA ratios of mysids held at 8°C were lower than those of mysids kept at 3°C (Fig. 3) and reached a final index ratio of ~1.5 after 3 weeks of fasting. It can only be speculated whether an extension of the experimental period would have further reduced the RNA/DNA ratios. At colder temperatures, RNA translational efficiency is lower than at higher temperatures (Pannevis and Houlihan 1992). Therefore, animals need higher concentrations of RNA to maintain their metabolic functions at colder temperatures. This might explain the higher RNA/DNA ratios observed in the animals fasted at 3°C for 21 d. Juinio *et al.* (1992) observed similar patterns and RNA/DNA values in American lobster. Animals held at 15°C had reached a RNA/DNA ratio of 1.4 after 20 d of fasting while animals held at 18°C or 21°C had reached ratios of 1.0. The relationship between temperature and RNA/DNA ratios after prolonged fasting suggests that the minimum index ratio will be a function of temperature: the ratio will be higher in animals experiencing colder conditions. However, it cannot be excluded that there is a common minimum RNA/DNA ratio for starved *Mysis* independent of temperature, which may simply take longer to be reach at lower temperatures.

The fact that there was no effect of fasting on the RNA/protein ratios of mysids after 11 d of fasting (Fig. 2) could be explained by the parallel decrease of RNA and protein during that time. Animals fasting for 21 d did show decreasing RNA/protein ratios. A similar pattern was observed for the protein/DNA ratios (Fig. 3). The effect of fasting on protein content clearly undermines the use of the RNA/protein ratio as an index of condition or growth rate (negative) for fasting mysids. However, the protein/DNA ratio would still provide an indication of cell size and condition. Clear and rapid results were obtained for the RNA/DNA ratio. Therefore, we recommend RNA/DNA for field assessments of the condition of juvenile mysids.

Field Versus Laboratory Estimates

Condition indices have to be calibrated before they can be applied to field situations. The field-caught *Mysis* showed the same nucleic acid ratios as *Mysis* collected at the same time but which were then fed *Artemia* nauplii for 3 weeks. Therefore, we can assume that the experimental animals used for our study were in good condition at the start of the experiment. Our study demonstrated that juvenile *Mysis* which are exposed to fasting conditions for 3 weeks have significantly reduced ratios of RNA/DNA (< 1.5–1.8) and that the degree of decline is related to temperature. Consequently, we conclude that field-caught animals having RNA/DNA ratios near these levels are demonstrating clear signs of metabolic stress. However, we emphasize that the ultimate cause of that stress would still have to be identified.

The invasion of the Great Lakes by exotic invertebrates (*Bythotrephes*, *Cercopagis*, and *dreissenids*) has altered the base and intermediate levels of the food webs. Nucleic acid ratios in *Mysis* should be monitored over the year to identify natural seasonal patterns as a background against which to evaluate and quantify the effects of such

wide-scale ecosystem perturbations. We suggest that mysid condition indices, such as RNA/DNA and protein/DNA ratios, could be used to help assess the overall impacts of such exotic invaders on the food web.

CONCLUSIONS

Hypertrophy plays a significant role in the growth of juvenile *Mysis*. Juvenile *Mysis* can survive for several weeks under fasting conditions and are well adapted to mobilize their metabolic reserves during periods of food deprivation. Variation in the nucleic acid ratios RNA/DNA, RNA/protein, and protein/DNA reflected the radical changes in the metabolic activity of fasting animals, and all these indices were significantly influenced by the water temperature. Since both RNA and protein concentrations are simultaneously changing in fasting *Mysis*, we recommend that only RNA/DNA and protein/DNA ratios should be used as indices of metabolism and condition in fasting animals. Juvenile *Mysis* showing RNA/DNA ratios < 1.5–1.8 can be clearly identified as metabolically-stressed animals. The response time of RNA to fasting ranged from 3 to 4 d at 8°C to between 4 and 11 d at 3°C. The final stable levels of these measures may not be reached for several weeks: the exact time will depend on the initial condition of the animals.

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