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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-