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Short communication

Effects of temperature on growth, adenosine phosphates, ATPase and cellular defense response of juvenile shrimp *Macrobrachium nipponense*

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Abstract

This study examined the effects of temperature on growth, adenosine phosphates, ATPase and cellular defense response of juvenile shrimps *Macrobrachium nipponense*. The results showed that specific growth rates in the temperature range 22–32 °C were significantly ($P<0.05$) greater than that at 16 and 20 °C, and were highest at 25 °C. The average (0.513±0.032) of hepatopancreas Na^+–K^+ ATPase activities in the temperature range 16–22 °C were enhanced 1.38-fold than that (0.216±0.069) of hepatopancreas Na^+–K^+ ATPase activities in the temperature range 25–32 °C ($P<0.05$). The adenylate energy charge (AEC) in muscle showed higher value ($P<0.05$) at 16 °C and 20 °C compared to in the temperature range 22–32 °C. ROIs level of abdominal muscle at 25 °C was the lowest ($P<0.05$). Moreover, SOD activity of abdominal muscle at 22–30 °C was higher ($P<0.05$) than that at 16 and 32 °C.

Keywords: Temperature; *Macrobrachium nipponense*; Adenosine phosphates; Cellular defense response

1. Introduction

Environmental fluctuations associated with seasonal climatic changes are of major importance in triggering adjustments in the physiology and behavior of aquatic organisms. Temperature is considered to be one of the most important physical factors influencing organisms, and the biological effects of these factors are complex and wide-ranging (Ponce-Palafox et al., 1997). Temperature change has striking effects on many physiological processes. For example, the effect on the rate of oxygen consumption is a convenient expression for the overall metabolic activity of an animal. As water temperature changes seasonally, physiological processes in the gills, kidneys and gut, which regulate body ion levels in fresh water fish, must continue to function effectively. This means that in temperate climates enzyme activities involved in epithelial transport have to be maintained over a 20–30 °C temperature drop from summer to winter (Packer and Garvin, 1998). A number of investigators have shown that the lower temperature results in fall of active gill ion uptake (Motais and Isaia, 1972). Due to its central role in ion balance, effects of temperature changes on gill Na^+–K^+ ATPase have been studied presently.

The effects of temperature stress and temperature acclimation on *Macrobrachium nipponense* have been

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described before (Yamane, 1995). The amount of catch on *M. nipponense* in Lake Biwa was controlled by the accumulated water temperature (Yamane, 1995). Energy budget of *M. nipponense* was affected by water temperature (Dong et al., 1994a). Responses to temperature stress may be related to an increased oxygen consumption rate (Dong et al., 1994b). As an estimated proportion, 2–3% of the oxygen consumed by aerobic cells is converted to oxygen radicals (O_2^-) and H_2O_2 (Sohal and Weindruch, 1996) and increasing tissue oxygen consumption will entail elevated rates of reactive oxygen species (ROS) production in mitochondria (Boveris and Chance, 1973). In crustaceans, the demonstration of a respiratory burst is quite recent. Bell and Smith (1993) demonstrated the generation of superoxide anions by haemocytes of the decapod *Carcinus maenas*. Muñoz et al. (2000) demonstrated the production of superoxide anions (O_2^-) by haemocytes of the white shrimp *Penaeus vannamei*. Earlier evidence indicates that the health of aquatic organisms is also linked to over production of ROS in their tissues (Di Guilio et al., 1989). Further, exposure of aquatic organisms to nitrite and ammonia-N (Wang et al., 2004, 2005), pesticides (Doyotte et al., 1997) and heavy metal ions (Dandapat et al., 1999) resulted in induction of oxidative stress.

Although not previously examined in *M. nipponense*, temperature difference in adenylate (ATP, ADP and AMP) levels, AEC have been noted in two crustaceans (Bamstedt and Skjoldal, 1976; Skjoldal and Bamstedt, 1976) and freshwater clams (Giesy and Dickson, 1981). Energy charge (EC) is an index of the amount of energy available to an organism from the adenylate pool. It is calculated from the measured concentrations of the three adenine nucleotides: adenosine triphosphate (ATP), adenosine diphosphate (ADP) and adenosine monophosphate (AMP), which are fundamental to the energy metabolism of all organisms (Atkinson, 1977). Many studies have demonstrated that certain ranges of AEC correlate with physiological condition. Energy charges between 0.8 and 0.9 are typical of organisms, which are actively growing and reproducing, usually under optimal conditions (Vetter and Hodson, 1982). Due to more loose regulations and particularly to the low efficiency of the enzyme AMP deaminase, invertebrates are able to survive to lower energy charge (0.3–0.4) than vertebrates (0.5–0.6) (Marazza et al., 1996). AEC has been extensively applied as a useful indicator of environmental stress (Barthel, 1984).

The major aim of this investigation is therefore to study the effects of temperature on growth, survival, phosphate adenylate levels, ATPase activity and cellular defense response of juvenile shrimp, *M. nipponense*, a commercially important species, found in the brackish and freshwater throughout China (from North China to Taiwan), Japan and Vietnam (Wang et al., 2001).

2. Materials and methods

The experimental shrimps, *M. nipponense* were collected from Bai Yangdian Lake, Hebei Province, China, on March 2001.

Juvenile shrimps were selected for the experiments and had a mean weight of 0.112±0.050 g and a length of 1.8±0.3 cm. They are at the intermolt stage.

In the laboratory, water temperatures were maintained at 16, 20, 22, 25, 27, 30 and 32±1 °C, respectively, using aquarium heaters. Groups of 50 shrimps were kept in 60×30×25-cm tanks at various temperatures. These tanks had recirculated water by means of an airlift. Each group was replicated. Each day, 25% of the water was replaced by a fresh water supply of the same temperature. The shrimps were fed with commercial flake shrimp food. An estimation of daily food consumption was obtained via a visual estimate of food remaining in the tanks before morning cleaning. Food was provided slightly in excess and was based on the previous days' consumption. To compensate for mortalities, the number of prawn days of feeding was calculated (based on survivors at each weighing) and used to calculate daily weight gain and feed intake for each prawn. Food conversion ratios (FCR) were calculated as the estimated dry weight of feed consumed per day (g) per prawn wet weight increase per day (g). Acclimatization lasted for 19 days, prawns were freeze-clamped in liquid nitrogen after prawns were weighed.

2.1. Adenylate extraction and adenylate determination

The nucleotides in shrimp muscles were extracted using a modified procedure of Zaroogian et al. (1982). Muscle samples (0.5 g) were homogenized with 4.5 ml 5% cold perchloric acid. After centrifugation at 9960×*g* at 4 °C, the supernatant was collected. The extract was stored at −80 °C until analysis.

The concentrations of ATP, ADP and AMP were determined using HPCE according to a procedure already described by Wang et al. (2001, 2003). Under the optimized conditions: 50 mM Na_2HPO_4+20 mM CTAB+1 mM EDTA (pH 8.0), −15 kV, 5 kPa for 6 s load and detection at 254 nm.

2.2. ATPase assay

The gills, muscles and hepatopancreas were excised rapidly from prawn freeze-clamped at liquid nitrogen