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Growth and physiological responses in the sea cucumber, *Apostichopus japonicus* Selenka: Aestivation and temperature

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ABSTRACT

Aestivation is an adaptation of the sea cucumber *Apostichopus japonicus* Selenka to high temperature, however, the causations and physiological responses of aestivation are not well understood. This study deals with the relationship between temperature and aestivation. Sea cucumbers were allocated into four treatments. In two treatments of temperature elevation, the ambient temperature gradually was increased from 16 °C to 26 °C linearly (treatment FA) or by a fluctuating temperature profile (treatment FB). Two control treatments maintained constant temperatures of 16 °C and 26 °C, and were designated as optimum temperature of growth and threshold of aestivation, respectively. During the 40-day experiment, body weight, oxygen consumption, daily food intake, catalase (CAT) and superoxide dimutase (SOD) activities and heat shock protein 70 (Hsp70) levels were determined periodically. When the temperature gradually increased from 16 °C to 26 °C, the body weight of the tested sea cucumbers decreased gradually. After the ambient temperature reached 26 °C, the tested sea cucumbers in treatments of FA and FB were reared at 26 °C for an additional twenty days. During this period, symptoms of aestivation appeared in the tested sea cucumbers. Activities of antioxidases and Hsp70 levels increased when the ambient temperature increased from 16 °C to 26 °C, and decreased when the temperature was kept at 26 °C. These results indicate that aestivation in *A. japonicus* is an adaptive strategy to reduce the production of reactive oxygen species (ROS) and denatured proteins which were induced at high temperature.

1. Introduction

Aestivation is a kind of special dormancy to high temperatures and occurs in many organisms, such as mollusks (land snail, Solomon et al., 1996), amphibians (frog, Withers and Guppy, 1996; Hudson et al., 2004), fish (lungfish, Chew et al., 2004) and sea cucumber (Li et al., 1996; Liu et al., 1996; Yang et al., 2005). Adaptations to stressful environments typically include adjustments at multiple levels, including behavioral, physiological and biochemical adaptations. The physiological and biochemical mechanisms of aestivation have been studied in some animals (anuran amphibians, Cowan and Storey, 1999; pulmonate land snails, Brooks and Storey, 1997).

Cellular stress responses including enhancement of activities of antioxidases, synthesis of molecular chaperones and proteases and activation of DNA repair systems are important in resisting thermal stress (Sørensen et al., 2003). As two primary antioxidases that are directly involved in the eliminating of reactive oxygen species (ROS), superoxide dimutase (SOD) and catalase (CAT) are qualified as main indices of the antioxidant defense (Wilhelm-Filho et al., 1993, 2001; Leiniö and Lehtonen, 2005). In *Apostichopus japonicus*, the activities of SOD and CAT increased significantly when the ambient temperatures changed daily in amplitude over ±4 °C. (Dong et al., 2008a). Tissue level of heat shock protein 70 (Hsp70) is qualified as a direct index of the heat shock response. It is well established that Hsps can be induced under various physical, chemical and biological stressors (Feder and Hofmann, 1999). A strong correlation between the cellular heat shock response, which is defined as cellular induction of proteins including Hsps under thermal stress, and the thermal tolerance of animals has also been reported (Parsell and Lindquist, 1993; Feder and Hofmann, 1999; Tomanek, 2002). The continuous activation of heat shock proteins might take precedent over the synthesis of other proteins, and a number of potentially deleterious effects of Hsp have also been reported (Feder, 1999; Dahlhoff et al., 2001). The high level of Hsp70 could result in growth retardance (Feder et al., 1992; Krebs and Feder, 1997; Feder and Hofmann, 1999; Viant et al., 2003) because of the high energy cost during the synthesis of Hsps (Somero, 2002). As described previously, Hsp70 play important roles in against thermal (Dong and Dong, 2008) and osmotic stresses (Dong et al., 2008b) in the sea cucumber *A. japonicus*.

Sea cucumber *A. japonicus* is a common echinoderm in Japan, Korea and north of China (Liao, 1997), and has high commercial value (Chen, 1990). The sea cucumber aestivates at high temperature annually (Li et al., 1996; Chang et al., 2003). Characters of aestivation in *A. japonicus* include fast, gut tract degeneration, weight loss and metabolic rate depression (Sloan, 1984; Li et al., 1996, 2002; Liu et al.,

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1996; Yang et al., 2005). Different threshold temperatures to aestivation (20–30 °C) in the sea cucumber had been reported in previous studies, depending on the location (Sui, 1989; Li et al., 1996) and body size (Liu et al., 1996; Yang et al., 2005). An et al. (2007) found that the aestivation of the sea cucumbers *A. japonicus* at summer might be in response to a shortfall in energy intake with respect to an increase of energy consumed in respiration at high temperature. However, the physiological changes during aestivation in the sea cucumber are still not well understood.

In its natural habitat, *A. japonicus* undergoes diel and seasonal temperature fluctuations. Daily fluctuating temperatures could significantly affect growth and metabolism of *A. japonicus* (Dong and Dong, 2006; Dong et al., 2006, 2008a). Therefore, it is important to study the effect of temperature fluctuation on the physiological responses of the sea cucumber during aestivation. A fluctuating temperature treatment (FB) was designed to simulate the diel fluctuation of temperature in the natural habitat.

In the present study, growth, oxygen consumption rate (OCR), daily food consumption, activities of antioxidases and Hsp70 levels were studied in two continuous temperature elevation courses to determine the physiological responses of the sea cucumber when ambient temperatures elevated gradually from the optimal growth temperature (16 °C) to the threshold temperature of aestivation (26 °C).

2. Materials and methods

2.1. Collection and maintenance of animals

Two-year old sea cucumbers were collected from Jiaonan Aquaculture Farm, Qingdao, P. R. China and acclimated at 16 °C for two weeks.

Seawater was filtered using a sand filter and the salinity was 28–30 ppt. One-half or two-thirds of the rearing water was exchanged by fresh equi-temperature seawater daily. Aeration was provided continuously, and the photoperiod was 12 h light: 12 h dark. The sea cucumbers were fed *ad libitum* pellets daily at 1300 h on a commercial formulated feed (22.9±0.2% crude protein, 2.1±0.0% fat, 34.7±0. 6% ash and 9.0±0.0% moisture, 10.6±0.0 kJ g^{-1} energy), which mainly contained powders of *Sargassum* spp., fish meal, sea mud, wheat, vitamin and mineral premixes (Liuhe Marine Tech. Cop., Qingdao, China).

2.2. Temperature treatments

In treatment FA, temperature increased from 16 °C to 26 °C at a rate of 0.5 °C per day, and then water temperature was maintained at 26 °C for 20 days (Fig. 1). In treatment FB, the amplitude of diel temperature fluctuation was 2 °C (temperature increased from 0600 to 1400, and decreased from 1400 to 0600), and the change of the mean temperature was the same as that in the treatment FA. Two constant temperature treatments of 16C (16 °C) and 26C (26 °C) were designed as controls.

Temperature of experimental aquaria was controlled by a laboratory-designed temperature control system (Dong et al., 2006). This system was composed of programmed temperature-controller, heater, refrigerator, recirculation pump and cold water reservoir. The fluctuating temperatures could be attained by pumping the cold water and heating the water alternately, controlled via the programmed temperature controller.

2.3. Growth

Sea cucumbers were kept in glass aquaria (450×250×350 mm). A total of 12 experimental groups (five sea cucumbers/group) were used. Three groups were randomly assigned to each treatment of 16C, 26C, FA and FB.

After a 24-h starvation, the initial wet weight measurements were taken within 1 min of removal from seawater and external water was removed from specimens by drying them on sterile gauzes (Dong et al., 2006). The mean initial weight of the sea cucumbers was 35.9±1.7 g (mean±S.E.), and there were no significant differences in the initial weights among the four treatments ($P>0.05$).The sea cucumbers were weighed every 10 days. During the whole experiment period, the daily food supplied was precisely weighed and recorded. The uneaten feed was collected by siphon, and was dried at 65 °C to constant weight.

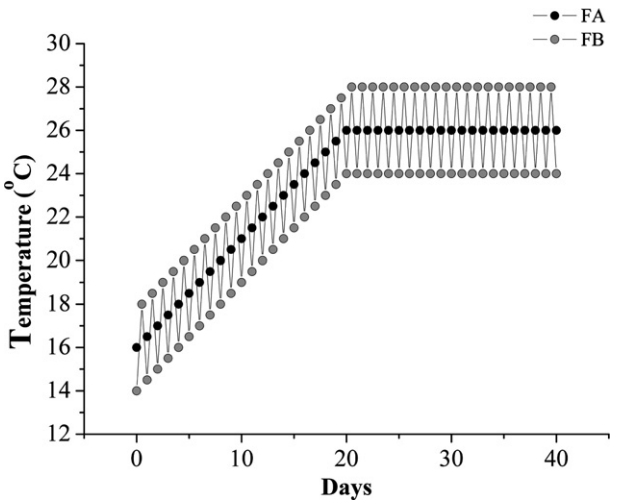

Fig. 1. Diagram of the temperature change mode of treatments of FA (–■–) and FB (–○–).

The specific growth rate (SGR) in terms of the wet weight was calculated as the following:

$$\mathrm{SGR}\left(\%\ \mathrm{day}^{-1}\right) = 100(\ln W_2 - \ln W_1)/D$$

where, W_2 and W_1 are the final and initial body weight of the sea cucumber (g), respectively; D is the duration of the experiment (day).

2.4. Oxygen consumption

Oxygen consumption rate (OCR) of the sea cucumbers, with a mean wet weight of 37.3±4.1 g, was measured every four days. Prior to the determination of oxygen consumption, sea cucumbers were starved for 24 h to reduce associated metabolic responses. In the two constant temperature treatments, there were three replicates and one blank control to correct for the respiration of bacteria in the water. In the treatments of FA and FB, there were four replicates and one blank control. The tested animals were put into a 3 L conical flask individually. When the sea cucumber became quiescent after 12 h, oxygen consumption was determined over 24 h and water in the conical flask was siphoned off every 8 h. Oxygen content of water samples was determined using the Winkler method (Strickland and Parsons, 1968).

Oxygen consumption rate (OCR) of the sea cucumber was calculated from the following equation (Omori and Ikeda, 1984):

$$\mathrm{OCR}\left(\mu\mathrm{g}\mathrm{O}_2\mathrm{h}^{-1}\mathrm{g}^{-1}\right) = (D_t V_t - D_0 V_0)/WT$$

where, D_t, changes of the oxygen content (μg O_2L^{-1}) before and after test in the test bottles; D_0, changes of the oxygen content (μg O_2L^{-1}) before and after test in the blank bottles; V_t, volumes of the test bottles (L); V_0, volumes of the blank bottles (L); W, wet weight of the sea cucumber (g); T, time duration (h).

2.5. Enzyme activity and Hsp70

A total of 16 experimental groups (five sea cucumbers/group) were assigned to treatments of 16C, 26C, FA and FB randomly. At Day 0, Day