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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.

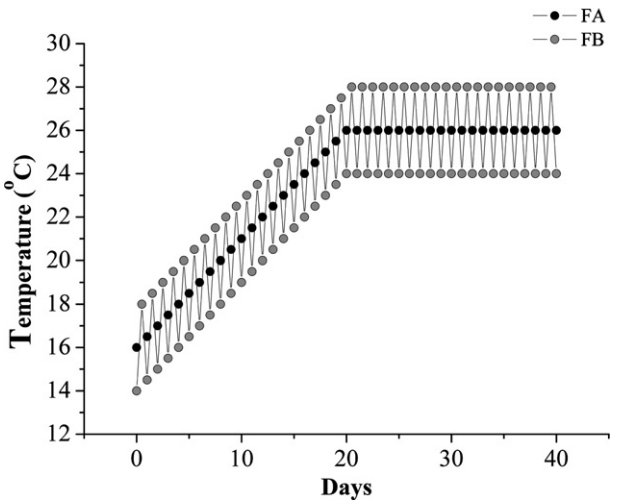

Fig. 1. Diagram of the temperature change mode of treatments of FA (–■–) and FB (–○–).

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

Table 1
Effect of different temperature treatments (16C, 26C, FA, FB) on body weight, viserosomatic index (VI) and respirosomatic index (RI) of the sea cucumber *Apostichopus japonicus* at different time points (Day 0, 10, 20, 30, 40)[a]

	Body weight		Viserosomatic index				Respirosomatic index			
	χ^2	*P*	df1	df2	*F*	*P*	df1	df2	*F*	*P*
16C	12.00	0.02	4	19	0.408	0.800	4	19	0.323	0.858
26C	11.47	0.02	4	19	156.706	<0.001	4	19	9.004	0.001
FA	6.13	0.19	4	19	97.184	<0.001	4	19	4.401	0.015
FB	9.87	0.04	4	19	162.923	<0.001	4	19	3.275	0.041

[a] Friedman Test was performed to analyze the difference in body weight (n=3) among different time points; the temporal changes of RI (n=4) and VI (n=4) were analyzed using one-way analyses of variance (ANOVA) followed by Post-Hoc Duncan Multiple Range Test. df1, degrees of freedom within groups; df2, total degrees of freedom.

10, Day 20, Day 30, Day 40, four sea cucumbers in each treatment were weighed. Their body wall, intestine and respiratory tree were quickly removed, weighed and frozen in the liquid nitrogen until further use.

The viserosomatic index (VI) and respirosomatic index (RI) were calculated using the following equation,

$$\mathrm{VI}(\%) = 100 \times W_i/W_2$$

$$\mathrm{RI}(\%) = 100 \times W_r/W_2$$

where, W_i is the weight of intestine (g); W_r is the weight of respiratory tree (g); W_2 is the final body weight of the sea cucumbers (g).

The preparation and measurement of enzymes were carried out as methods described by Dong et al. (2008a,b). SOD (EC 1.15.1.1) activity was measured with a modified xanthine/xanthine oxidase method (McCord and Fridovich, 1969) and CAT (EC 1.11.1.6) activity was measured according to Goth (1991).

Levels of Hsp70 were quantified following the methods of Dong et al. (2008a). Specimens were treated with homogenization buffer in the Cell Lysis Kit (BBI, Canada) containing protease inhibitor cocktail. There were three replicates per treatment. Specimens were homogenized on ice and centrifuged (10,000 *g*, 10 min). The supernatant fraction was collected and kept at −70 °C until use. Protein was determined as described by Lowry et al. (1951) with bovine serum albumin as standard.

Gel electrophoresis of protein extracts was performed on 10% polyacrylamide gels (PAGE) according to Laemmli (1970). Protein samples (equal amount of 20 μg total protein) were subjected to gel electrophoresis in the presence of Dithiothreitol (DTT). Semi-dry electrotransfer was performed according to Kyhse-Andersen (1984) onto PVDF-Immobilon membranes (0.45 μm, Millipore). Membranes were blocked and incubated with monoclonal anti-Hsp70 antibody (H5147, Sigma, USA) (diluted 1:1000) for 2 h at 37 °C, and then the membranes were incubated for 1 h with anti-mouse IgG (Horseradish peroxidase conjugatedm, HRP). The western blot was developed using ECL reagents (Amersham, USA), and exposed to X-ray film (Kodak, USA). Band intensity was quantified using GeneTools software (Syngene, USA).

Hsp70 level in body wall of sea cucumber sample (5 μg total protein/lane), which was subjected to an acute temperature enhancement (from 10 to 30 °C) for 1 h, was designed as a standard for normalization. For the sample used as standard, the sea cucumber was put into a 2 L glass beaker from 10 °C water. The temperature of the water in the beaker was adjusted to 30 °C before the sea cucumber was put into the beaker. After exposed at 30 °C for 1 h, the sea cucumber was transferred to 10 °C water for 2 h recovery. The tissue sample was prepared by the method mentioned above. Hsp70 levels in this study were shown as values relative to the level of the standard sample (Relative unit, RU).

2.6. Statistics

All analyses were performed using the SPSS for Windows (Version 11.0) statistical package. Data were tested for homogeneity of variances using Mauchly's Test of Sphericity. OCR was analyzed by repeated measures analysis of variance. When the assumption for parametric analysis was violated, a nonparametric test (Friedman Test) was performed to analyze the difference of the body weight among different time points. The temporal changes of RI, VI, enzyme activities and Hsp70 levels were analyzed using one-way analyses of variance (ANOVA) followed by Post-Hoc Duncan Multiple Range Tests. The difference of SGR among different treatments was analyzed using ANOVA. Differences were considered significant if $P<0.05$.

3. Results

3.1. Growth

The temporal change patterns of body weight were different in the four treatments during the 40-day trial (Table 1, Fig. 2). The body weights in 16C sea cucumber increased gradually, but kept decreasing at treatment of 26C and FB. Although there was no statistical difference due to the large variations of body weight in the treatment of FA, the body weight of the sea cucumber also showed a decreasing trend.

After the 40-day experiment, SGR in 16C sea cucumber was significantly higher than that in treatments of 26C, FA and FB (ANOVA: $F(3, 11)=7.52$, $P=0.01$), and there was no significant difference in SGR among treatments of 26C, FA and FB (16C, 0.58±0.28% day^{-1}; 26C, −0.46±0.07% day^{-1}; FA, −0.34±0.18% day^{-1}; FB, −0.30±0.07% day^{-1}, mean±S.E., n=3).

3.2. Daily food consumption

The daily food intake among the four treatments was different (Fig. 3). Individuals in treatment 16C kept eating actively during the whole experimental period. On the other hand, the food consumption in 26C sea cucumbers decreased gradually, and dropped to zero at Day 18. The daily food intake fluctuated in treatments of FA and FB, and the daily food consumption in the FB sea cucumber was appreciably higher than that in the FA sea cucumber. An obvious decrease of food consumption in treatments of FA and FB occurred from Day 35 to the end of the experiment.

3.3. Viserosomatic (VI) and respirosomatic index (RI)

There were no significant differences in VI and RI in 16C sea cucumber during the 40-day trial (Table 1, Fig. 4). In treatments of 26C, FA and FB, VI and RI decreased gradually during the whole experimental period.

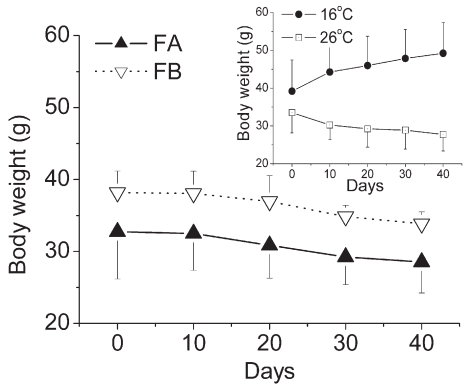

Fig. 2. Mean (±1 S.D.) of body weight in the sea cucumbers *Apostichopus japonicus* at Day 0, Day 10, Day 20, Day 30 and Day 40 under the two temperature elevation treatments (FA and FB). Inset: Mean (±1 S.D.) of body weight in the sea cucumbers *Apostichopus japonicus* at Day 0, Day 10, Day 20, Day 30 and Day 40 under the two constant temperature (16 °C, 26 °C), n=3 in all treatments.

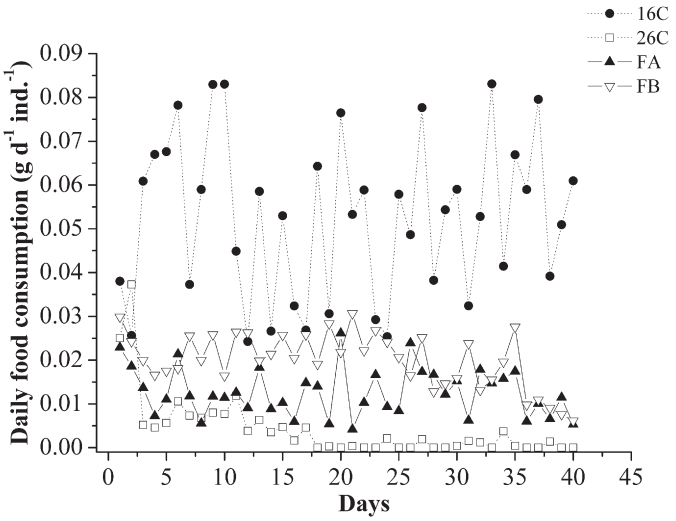

Fig. 3. The daily food consumption of the sea cucumbers *Apostichopus japonicus* in the four treatments in a 40-day trial. During the whole experiment period, the daily food supplied was precisely weighed and recorded. The uneaten feed was collected by siphon, and was dried at 65 °C to the constant weight.

3.4. Oxygen consumption

During the whole experimental period, OCR in 16C sea cucumber was stable and maintained at a low level (Fig. 5). On the other hand, the OCR in 26C sea cucumber decreased at the beginning of the experiment. The OCRs of FA and FB sea cucumbers increased with increasing temperature, and then decreased. The maximum values of OCR in treatments of FA and FB occurred at Day16 and Day 28, respectively. Repeated measure analysis of variance showed that the OCRs of sea cucumber among different treatments were significantly different: FA>FB>26C>16C (Table 2).

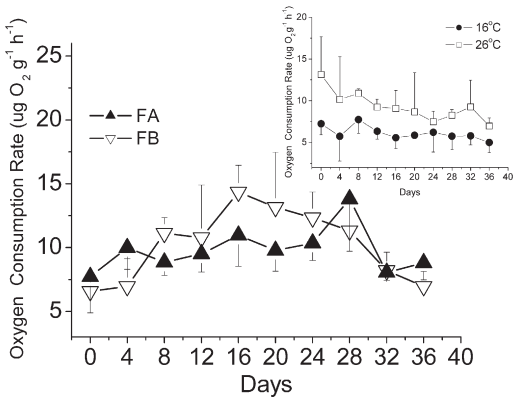

Fig. 5. Effects of different temperatures on the oxygen consumption rate (OCR) of the sea cucumber *Apostichopus japonicus* at the two temperature elevation treatments (FA and FB). Inset: the temporal pattern of OCR in the sea cucumber reared at 16C (16 °C) and 26C (26 °C). The OCRs of the sea cucumbers were measured every four days. Values are mean±1 S.D., n=3 specimens in the two constant temperature; n=4 specimens in treatments FA and FB.

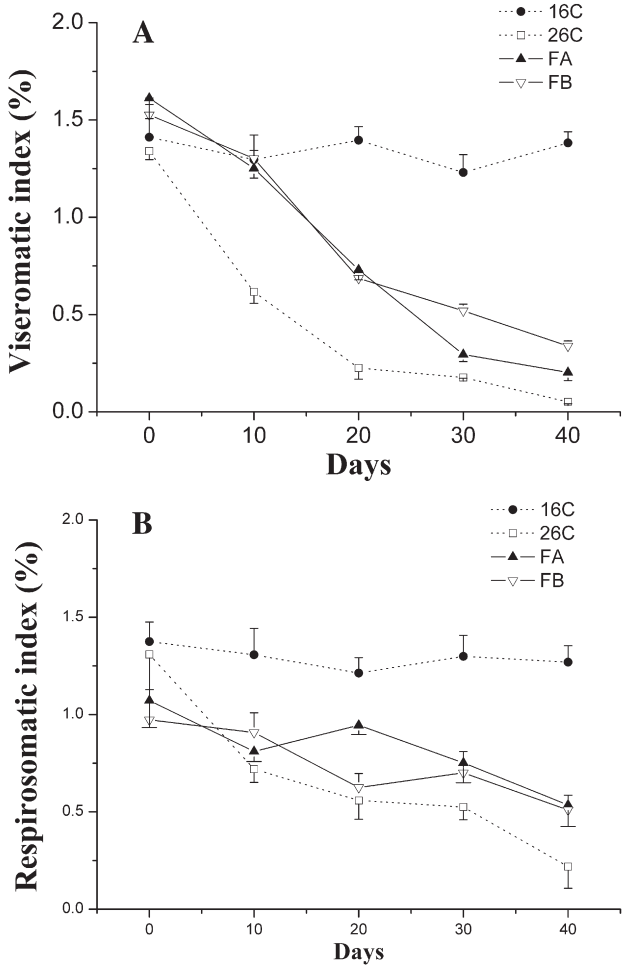

Fig. 4. Viserosomatic index (A) and respirosomatic index (B) of sea cucumber *Apostichopus japonicus* in the four temperature treatments in a 40-day trail. Values are mean±1 S.E., n=4.

3.5. Antioxidant enzymes

CAT and SOD activities of 16C sea cucumber remained at low levels, and there were no significant differences at different time points (Table 3).

In the 26C treatment, activities of SOD and CAT decreased gradually. Temporal patterns of the two antioxidases were tissue-specific in treatments of FA and FB. The following was the depiction of these patterns in detail. The statistical results are shown in Table 3.

3.5.1. Respiratory tree

In the 26C treatment, CAT activity decreased gradually, and SOD activity decreased from Day10 (Fig. 6). In treatment FA, CAT activity increased gradually to Day 30, and then decreased. In FB, the maximum of CAT activity was found at Day 20. In treatments of FA and FB, the SOD activity increased gradually, and the maximum occurred at Day 30 and Day 40, respectively.

3.5.2. Intestine

The activities of CAT and SOD in treatment 26C decreased initially and remained stable after Day 20 (Fig. 7). The maximum activity of CAT and SOD in treatment FA occurred at Day 30, and the maximum activity of the two enzymes in treatment FB occurred at Day 40.

3.5.3. Body wall

In treatment 26C, the activities of the two enzymes increased, and then decreased gradually (Fig. 8). In treatments of FA and FB, the maximum activity of SOD and CAT occurred at the end of the experiment.

Table 2
Effect of temperature treatments (16C, 26C, FA, FB) on the oxygen consumption rate (OCR) of the sea cucumber *Apostichopus japonicus* at different time points (Day 0, 4, 8, 12, 16, 20, 24, 28, 32, 36)[a, b]

Source	Type III sum of squares	df	Mean square	F	P
Time	113.838	9	12.649	2.347	0.020
Treatment	330.762	3	110.254	18.06	0.000
Time×Treatment	351.647	27	13.024	2.416	0.001

[a] Data were analyzed by repeated measure analysis of variance.
[b] Treatments of 16C, 26C, n=3; Treatments of FA, FB, n=4.

Table 3
Effect of temperature treatments (16C, 26C, FA, FB) on the catalase (CAT), superoxide dismutase (SOD) activities and Hsp70 level in respiratory tree (R), intestine (I) and body wall (B) of the sea cucumber *Apostichopus japonicus* at different time points (Day 0, 10, 20, 30, 40)[a]

		CAT				SOD				Hsp70			
		df1	df2	*F*	*P*	df1	df2	*F*	*P*	df1	df2	*F*	*P*
16C	R	4	14	1.25	0.35	4	14	2.17	0.15	4	14	1.59	0.25
	I	4	14	0.74	0.58	4	14	1.01	0.45	4	14	0.07	0.99
	B	4	14	1.64	0.24	4	14	3.04	0.07	4	14	0.44	0.78
26C	R	4	14	183.70	0.00	4	14	28.63	0.00	4	14	15.01	0.00
	I	4	14	104.55	0.00	4	14	32.49	0.00	4	14	19.72	0.00
	B	4	14	18.97	0.00	4	14	7.436	0.01	4	14	8.12	0.00
FA	R	4	14	8.02	0.04	4	14	11.48	0.00	4	14	25.82	0.00
	I	4	14	20.59	0.00	4	14	69.11	0.00	4	14	33.29	0.00
	B	4	14	39.60	0.00	4	14	23.35	0.00	4	14	21.10	0.00
FB	R	4	14	22.44	0.00	4	14	14.86	0.00	4	14	27.42	0.00
	I	4	14	13.21	0.00	4	14	16.65	0.00	4	14	21.75	0.00
	B	4	14	35.74	0.00	4	14	15.96	0.00	4	14	17.41	0.00

[a] The temporal changes of enzyme activities, and Hsp70 levels were analyzed using one-way analyses of variance (ANOVA) followed by Post-Hoc Duncan Multiple Range Tests. $n=3$ in all treatments. df1, degrees of freedom within groups; df2, total degrees of freedom.

3.6. Hsp70

In the three tissues, Hsp70 levels in 16C sea cucumber were maintained at a low level, and there were no significant differences among different time points (Table 3, Fig. 9). In treatment 26C, Hsp70 level decreased gradually in the respiratory tree. But in intestine and body wall, Hsp70 level increased at the beginning of the experiment, and then decreased. In treatments of FA and FB, Hsp70 levels increase gradually, and the maximum values occurred at the end of the experiment.

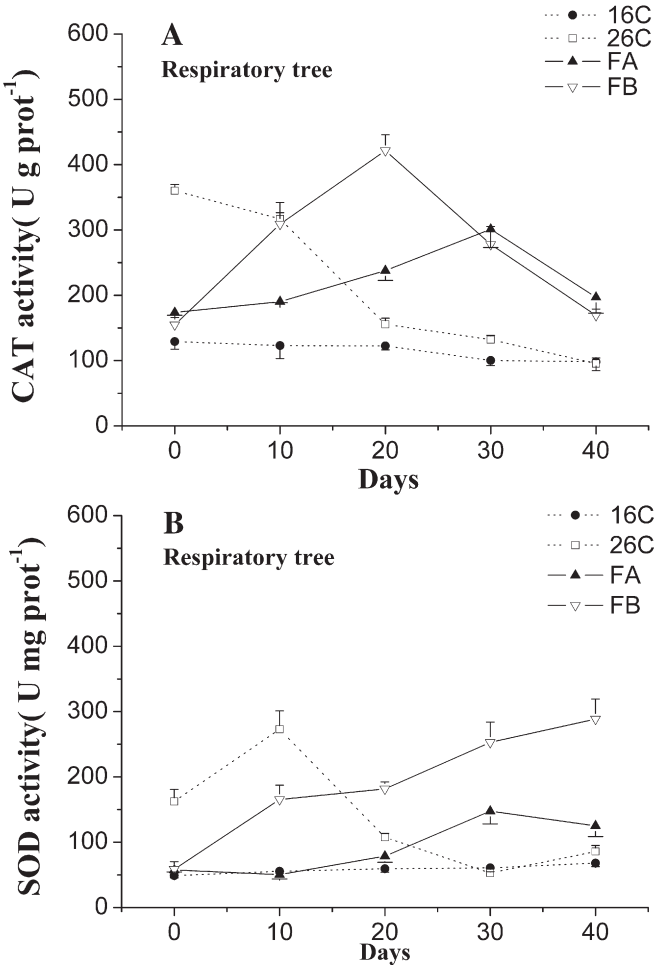

Fig. 6. Effects of different temperatures on CAT (A) and SOD (B) activities in the respiratory trees of sea cucumber *Apostichopus japonicus*. Values are mean ± 1 S.E., $n=3$ in all treatments.

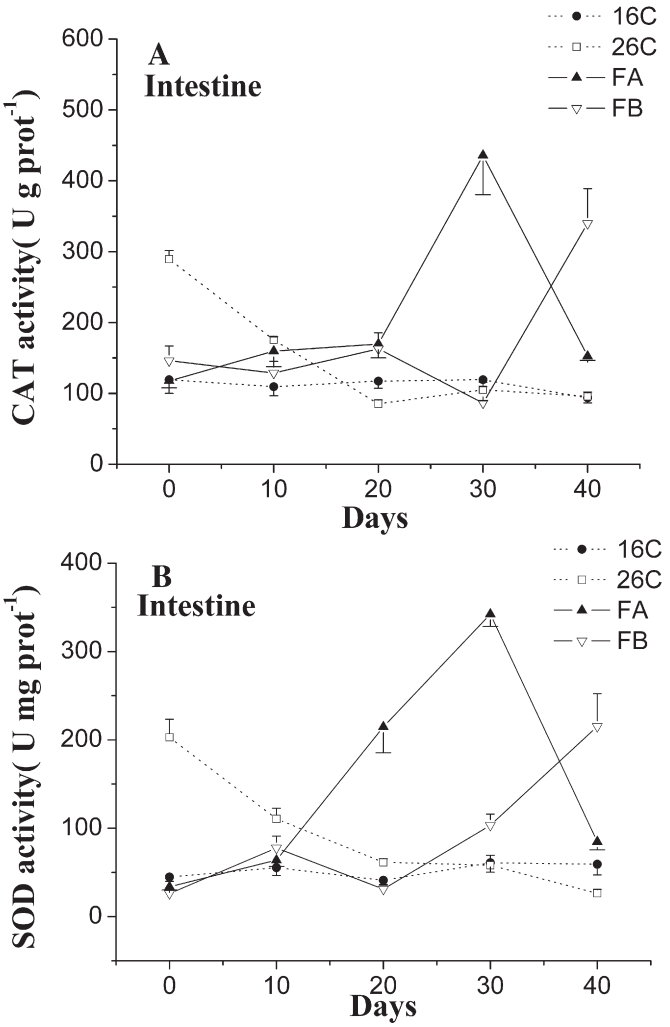

Fig. 7. Effects of different temperatures on CAT (A) and SOD (B) activities in intestines of the sea cucumber *Apostichopus japonicus*. Values are mean ± 1 S.E., $n=3$ in all treatments.

4. Discussion

Water temperature is one of the most important ecological factors that affect growth and physiological processes in aquatic eurytherms. Previous reports showed that empirically *A. japonicus* could grow at a temperature range of 10–20 °C (Sui, 1990; Yu and Song, 1999; Chen, 2004; Yang et al., 2005). Dong et al. (2006) found that the optimum temperature for growth in *A. japonicus* was 15–18 °C. Therefore, 16 °C was chosen as the optimum temperature in the present study. According to the studies of Chen (2004) and Yang et al. (2005), 26 °C was chosen as the threshold temperature to aestivation.

In the present study, the sea cucumbers in treatment 16C kept feeding during the whole period, and the body weight of 16C sea cucumbers increased gradually. The activities of antioxidases and Hsp70 level of the sea cucumbers reared at 16 °C maintained at low levels. These results indicate that the temperature of 16 °C is favorable for the growth of *A. japonicus*.

Symptoms of aestivation, such as fast, intestine degeneration, weight loss and metabolic rate depression, were observed in the sea cucumbers reared at 26 °C. The food consumption in the 26C sea

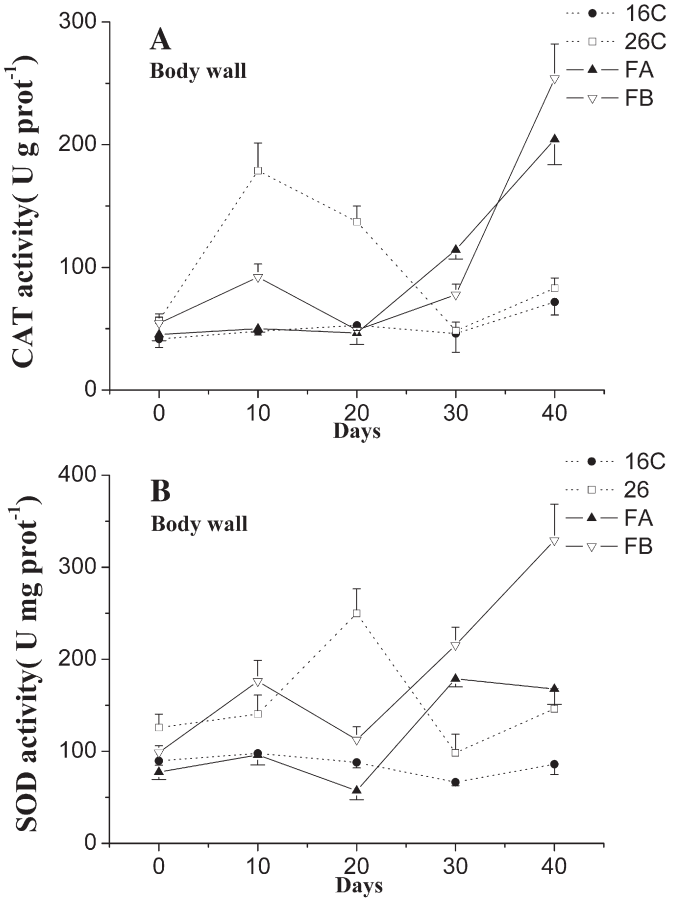

Fig. 8. Effects of different temperatures on CAT (A) and SOD (B) activities in body walls of sea cucumber *Apostichopus japonicus*. Values are mean±1 S.E., $n=3$ in all treatments.

cucumber decreased gradually, and dropped to zero at Day 18. After the 40-day trail, intestines of the 26C sea cucumber degenerated severely. The body weight and OCR in the 26C individuals gradually decreased during the whole experimental period. These results indicate that the sea cucumbers reared at 26 °C entered a state of aestivation and in this state overall rates of process were low.

In treatments of FA and FB, the OCRs increased initially and then decreased gradually. After the 40-day trial, the final body weights in treatments of FA and FB were lower than the initial body weights, and there was no significant difference in the SGRs among treatments of 26C, FA and FB. This evidence indicates that the growth of the sea cucumbers in treatments of FA and FB is retarded. Food consumption decreased after Day 35 and remained at low level for five days in treatments of FA and FB. At the end of the experiment, intestines of the sea cucumbers reared at treatments of FA and FB began to degenerate. These results indicate that the sea cucumbers in treatments of FA and FB have entered a state of aestivation though it was only at the beginning phase. These results also indicate that when the ambient temperature reaches the threshold of aestivation, *A. japonicus* does not aestivate immediately until a certain amount of accumulate temperature is achieved.

High temperature could initially upregulate the antioxidase activities, which kept decreasing when the sea cucumbers entered state of aestivation. In treatments of FA and FB, the activities of CAT and SOD were significantly higher than those in treatment 16C when the rearing temperature increased from 16 °C to 26 °C. In treatment of 26 °C, the activities of CAT and SOD in all tissues decreased with the duration of the experiment period (Figs. 6–8). The changes of CAT and SOD activities were related to the oxygen consumption rate. An enhancement of the tissue oxygen consumption increases the production of reactive oxygen species (ROS). Oxidative stress occurs when the ROS generation rate exceeds that of its removal (Sies, 1986). The deleterious effects of ROS include damages to proteins, DNA, and peroxidation of unsaturated lipids in cell membranes. The oxidized or nitrosylated products of ROS attack have been shown to decrease biological activity, leading to loss of energy metabolism, cell signaling, transport, and other major functions. These altered products also are targeted for proteosome degradation, further decreasing cellular function. Accumulation of such injuries ultimately leads the cell to die through a necrotic or apoptotic mechanism (Vincent et al., 2004). Oxidative damage is counteracted by antioxidant defense systems and repair mechanisms. SOD and CAT play

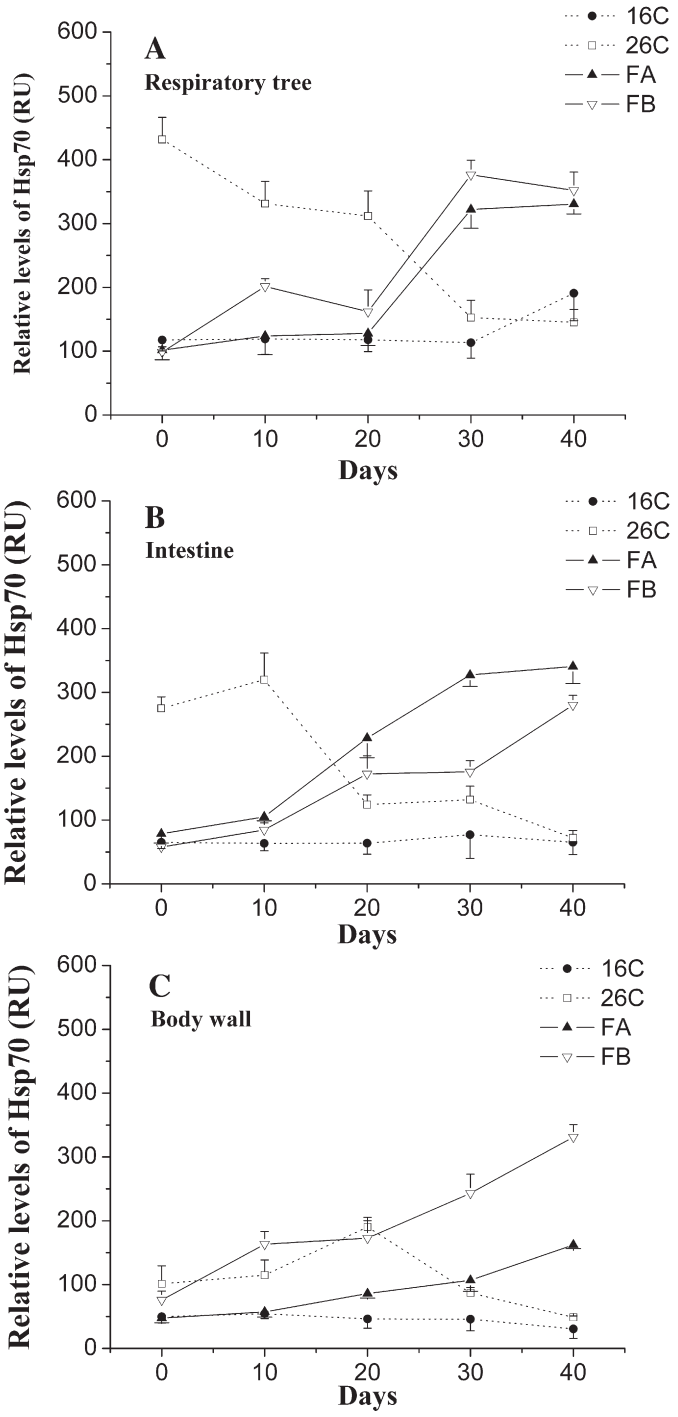

Fig. 9. Relative levels of heat shock protein 70 expression in respiratory trees (A), intestines (B) and body walls (C) of the sea cucumber *Apostichopus japonicus* under two constant temperatures (16 °C, 26 °C) and temperatures elevation treatments (FA and FB). Values are mean±1 S.E., $n=3$ specimens for all data points.

important roles in cellular antioxidants, and are directly involved in removing of ROS (Hermes-Lima et al., 1998; Pörtner, 2002; Bagnyukovaa et al., 2003). The changes of SOD and CAT activities during aestivation in *A. japonicus* indicate that the decrease of OCR is an adaptive response to high temperature in order to reduce the accumulation of ROS and related deleterious effects.

As a molecular chaperone, Hsp70 assists the refolding of stress-denatured cellular proteins and prevents these proteins from aggregating in the cell (Parsell and Lindquist, 1993). The Hsp70 levels increased with the increasing temperature in treatments of FA and FB. This result further indicates that the temperature of 26 °C is beyond the normal temperature limit for *A. japonicus*. At temperatures outside the thermal optimum, some proteins lose their structures and activities, leading to the expression of heat shock proteins (Hsps) to refold denatured proteins and prevent further aggregation and precipitation (Morimoto and Santoro, 1998; Feder and Hofmann, 1999). These pathways are energetically costly, suggesting a tradeoff between thermal tolerance and metabolic output (Somero, 2002). The down-regulation of Hsp70 indicates that a low level of denatured proteins occurred in the sea cucumber during aestivation. It may also be the case that there are denatured proteins during aestivation, but that they are not corrected or removed until the sea cucumbers returns to the suitable temperature and Hsp70 is expressed. Therefore, aestivation may be partly interpreted as an adaptive strategy of the sea cucumber to reduce energy cost and damage of protein denaturation at high temperature.

At the treatment of 26C, the maximum values of Hsp70 occurred at Day 0, Day 10 and Day 20 in the respiratory tree, intestine and body wall, respectively. The difference in the levels of Hsp70 might be due to the difference of the sensitivity to thermal stress among different organs (Koban et al., 1991; Wood et al., 1999; Zielinski and Pörtner, 2000; Selvakumar and Geraldine, 2005; Trenzado et al., 2006). The physiological responses of the sea cucumbers were slightly different between treatments of FA and FB, which might due to the changes of metabolism, enzyme activities and Hsp70 level at the fluctuating treatment compared to those in the constant temperature (Dong et al., 2006, 2008a,b).

In conclusion, the sea cucumber *A. japonicus* enters a state of aestivation when the ambient temperature is maintained at 26 °C. During the process of aestivation, oxygen consumption, activities of antioxidases and Hsp70 level increase initially, and then decrease. The decrease of oxygen consumption during aestivation is a kind of adaptive strategy for the sea cucumber to reduce the ROS, denatured proteins and related energy costs.

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