Aquacult Int (2009) 17:459–467
DOI 10.1007/s10499-008-9216-4

A high-performance temperature-control scheme: growth of sea cucumber *Apostichopus japonicus* with different modes of diel temperature fluctuation

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Received: 20 May 2008 / Accepted: 29 August 2008 / Published online: 24 September 2008

Abstract The effects of four modes of diel temperature-fluctuation with two designated fluctuating temperatures (15 ± 3°C and 18 ± 3°C) on the growth and energy budget of young sea cucumber, *Apostichopus japonicus* Selenka, were studied to develop a highly efficient temperature-control scheme for aquaculture of the species. Sea cucumbers with a mean wet body weight of 8.0 ± 1.2 g (mean ± SD) were allocated to each treatment randomly with five replicates. After a 38-day trial, specific growth rate (SGR) and food conversion efficiency (FCE) decreased with increasing temperature in constant-temperature treatments. Among the four modes of temperature fluctuation, SGR of sea cucumbers reared under a mode which simulated the natural fluctuation of the temperature (mode C) of seawater was significantly higher than that of sea cucumbers reared at the corresponding constant temperatures. This enhancement of growth rate by use of mode C was attributed to higher FCE and lower energy allocated to respiration and feces. In large-scale culture, a temperature-control mode designed based on mode C could enhance not only growth but also efficiency of food utilization by the young sea cucumber.

Keywords Energy budget · Temperature-fluctuation mode · Growth · Sea cucumber

Introduction

As a commercially important echinoderm in China, sea cucumber *Apostichopus japonicus* Selenka has become an increasingly important aquaculture species in the last decade. The total area devoted to sea cucumber farming has reached 10,000 ha in China, and output of the dried *A. japonicus* (beche-de-mer) reached 5,800 tons in 2000 (Chen 2004). As reported after previous studies, water temperature is an important physical factor affecting the metabolism (Yang et al. 2005), growth, and physiological performance of the sea

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cucumber (Dong et al. 2005). The optimum temperature for growth of young *A. japonicus* is between 15 and 18°C (Liao 1997) and metabolism increases with increasing temperature until the sea cucumber enters a state of estivation (Ji et al. 2008). Compared with constant temperature, fluctuation of temperature can affect growth of sea cucumber dramatically. Appropriate mean temperatures and amplitude of temperature fluctuation can enhance the growth of sea cucumber and improper designs can significantly retard its growth (Dong et al. 2006). Besides the mean temperature and thermal amplitude, the mode of temperature fluctuation is another important aspect of temperature fluctuation design (Alderdice 1976; Spigarelli et al. 1982) and the effects of temperature-fluctuation mode on growth of the sea cucumber are still not well understood.

Conventionally, young sea cucumbers are reared indoors during their first year. In winter, the temperature of natural seawater is often below 5°C in the aquaculture region of *A. japonicus*. It is, therefore, necessary to heat the rearing water to satisfy the requirements of the young sea cucumber. In order to develop a highly efficient temperature-control scheme, in this study we studied the responses of *A. japonicus* to different modes of temperature fluctuation by measuring specific growth rate (SGR) and energy budget.

Materials and methods

Source of animals

Test juvenile sea cucumbers were sampled at 15°C from Zhen-dong Aquaculture Corporation, Wei-hai, Shandong Province, P.R. China.

Acclimation and rearing conditions

Prior to the experiment, sea cucumbers were allocated to several fiberglass tanks with natural seawater and continuous aeration, and acclimated at 15°C for 10 days.

Temperature was controlled by the methods of Dong et al. (2006). For constant above-room-temperature treatments, the temperature of the water bath was regulated by use of a thermostat, which controlled the on/off switch of a 1,500 W electric heater. For fluctuating and below-room-temperature treatments, the temperature of the water bath was regulated by a laboratory-designed temperature-control system. This system was composed of a programmed temperature controller, a heater, a refrigerator, a recirculation pump, and a cold water reservoir; temperature control was achieved by alternately pumping cold water and heating the water, controlled by the programmed temperature-controller. Aeration was provided continuously and one-third of the water volume in all the experiment tanks was exchanged every day. Dissolved oxygen was maintained above 4.0 mg l^{-1}. A simulated natural photoperiod cycle of 12 h light:12 h dark was used. During acclimation, juvenile sea cucumbers were fed twice a day (0800 and 1800) with pieces of formulated feed made from a mixture of commercial feed (Liuhe Marine Tech., Qingdao, China) and sodium alginate. The composition of the feed was: 17.38 ± 0.22% crude protein, 1.67 ± 0.14% crude lipid, 22.89 ± 0.25% ash, and 9.31 ± 0.52% moisture; energy per unit was 8.55 kJ g^{-1}.

Experimental design and procedure

After 10-day acclimation and 24 h starvation, the initial body wet weights of the sea cucumbers were measured individually. Then 55 individuals (8.0 ± 1.2 g) were randomly

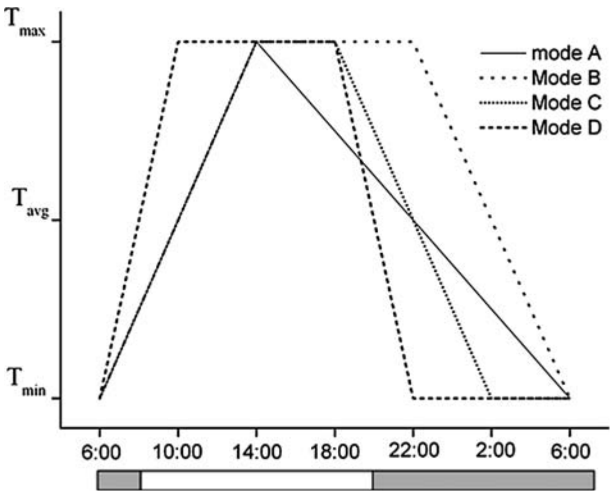

Fig. 1 Diagram of the four modes of diel temperature fluctuation. The photoperiod regime is depicted by the *horizontal white* (light period) and *black* (dark period) bars. T_{max}, T_{avg}, and T_{min} represent the maximum, average, and minimum temperatures of the designed temperature fluctuations

selected and dried at 65°C to constant weight to measure the initial energy content. A total of 275 individuals were randomly assigned to 55 aquaria filled with 30 l seawater (450 × 250 × 350 mm). Five individuals were allocated to each aquarium. Four modes (A, B, C and D) at two mean temperatures (15 ± 3°C and 18 ± 3°C) of diel fluctuating temperature (15A, 15B, 15C, 15D, 18A, 18B, 18C, and 18D) were designed (Fig. 1). Three constant-temperature treatments (15, 18, and 21°C) were designed as constant temperature controls. There were five replicates in each treatment. No significant difference of initial body weight was found among the treatments ($P > 0.05$).

During the course of the experiment the daily supply of feed was precisely weighed and recorded. Feces were collected by siphon in time to avoid decomposition. Collected uneaten feed and feces were dried at 65°C and kept for further analysis. After the 38-day trial, all the specimens were weighed after 24 h starvation and dried at 65°C for 48 h for analysis of energy and nitrogen content.

Energy determination and estimation of energy budget

The energy content of the food, sea cucumber bodies, feed, and feces were measured with a Parr (USA) 1281 oxygen bomb calorimeter. The energy budget was calculated by use of the equation (Carfoot 1987; An et al. 2007):

$$C = G + F + U + R$$

where C is the energy consumed in food, G the energy used for growth, F the energy lost in feces, U the energy lost in excretion, and R the energy used for respiration. The values of C and F can be calculated from the weight of the samples of food intake, feces weight, and their energy content per gram. G can be calculated by use of the equation:

$$G = (F_w \times E_f) - (I_w \times E_i)$$

where F_w and I_w are the final body weight and initial body weight, respectively, of the sea cucumbers and E_f and E_i are, respectively, the energy content per gram of final body and initial body of the sea cucumbers.

The nitrogen content of the sea cucumber bodies, food, and feces was measured with an Elementar (Germany) VarioEL III elemental analyzer. Estimation of U was based on the nitrogen budget equation (Levine and Sulkin 1979):

$$U = (C_N - G_N - F_N) \times 24{,}830$$

where C_N is the nitrogen consumed in food, F_N the nitrogen lost in feces, G_N the nitrogen deposited in the body, and 24,830 is the energy content (J g^{-1}) of excreted nitrogen.

The value of R was calculated using the energy budget equation:

$$R = C - G - F - U$$

Data analysis

Specific growth rate in terms of weight (SGR_w) and energy (SGR_e), feed intake (FI), and food conversion efficiency (FCE) in terms of weight (FCE_w) and energy (FCE_e) were calculated as:

SGR_w (% day^{-1}) $= 100 \times (\ln W_2 - \ln W_1)/D$
SGR_e (% day^{-1}) $= 100 \times (\ln E_2 - \ln E_1)/D$
FI (% body weight day^{-1}) $= 100 \times F/[D \times (W_2 + W_1)/2]$
FCE_w (%) $= 100 \times (W_2 - W_1)/F$
FCE_e (%) $= 100 \times (E_2 - E_1)/F_e$

where W_2 and W_1 are, respectively, the final and initial wet body weight of the sea cucumbers, E_2 and E_1 the final and initial body energy, respectively, of the sea cucumbers, D the duration of the experiment, F the total food taken by sea cucumbers during the whole experiment, and F_e the total energy taken by sea cucumbers from food during the whole experiment.

The data were analyzed using the SPSS for Windows statistical package (Version 10.0; SPSS, Chicago, IL, USA). Inter-treatment differences of SGR_w, SGR_e, FI, FCE_w, and FCE_e were analyzed by one-way ANOVA followed by post-hoc Duncan multiple-range tests. Differences were considered significant if $P < 0.05$.

Results

Growth

Among constant-temperature treatments, the relationship between SGR (SGR_w and SGR_e) and temperature (T) (from 15 to 21°C) could be described by the regression equations:

$SGR_w = 1.043 - 0.032T$ ($R^2 = 0.704$, $P < 0.01$ $n = 15$)
$SGR_e = 0.531 - 0.013T$ ($R^2 = 0.610$, $P < 0.01$ $n = 15$)

According to these equations, the growth of sea cucumber *A. japonicus* decreased with increasing temperature when the temperature was above 15°C.

The SGR_w in treatments 15C and 18C were significantly higher than in the other treatments at the same average temperature ($P < 0.05$) (Fig. 2a). The SGR_e of the sea

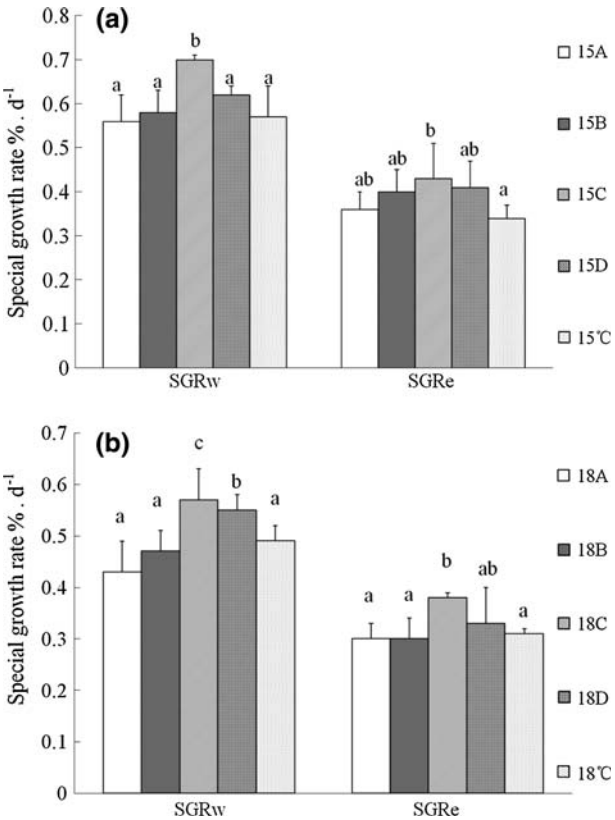

Fig. 2 The effects of patterns of diel fluctuating temperature on the SGR_w and SGR_e of the sea cucumber, *Apostichopus japonicus* for temperature regimes (**a**) 15 ± 3°C and (**b**) 18 ± 3°C. Histograms sharing a common letter are not significantly different ($P > 0.05$)

cucumbers in the 18C treatment was significantly higher than that in the other treatments except in the 18D treatment ($P < 0.01$) (Fig. 2b).

Food intake (FI) and food conversion efficiency (FCE)

There was no significant difference in FI among different modes of temperature fluctuation and constant temperatures ($P > 0.05$).

At a mean temperature of 15°C, FCE_w and FCE_e of the sea cucumbers in treatment 15C was significant higher than that of the sea cucumbers in treatments 15A, 15B, 15D, and 15°C constant temperature. At a mean temperature of 18°C, FCE_w and FCE_e for treatment 18C were significantly higher than for the other treatments ($P < 0.05$) (Fig. 3).

Energy allocation

The energy budget for the tested sea cucumbers changed among the different temperature regimes (Table 1). At a mean temperature of 15°C, the ratio G/C in treatment 15C was significantly higher than that in the other modes. The ratio F/C in treatment 15C

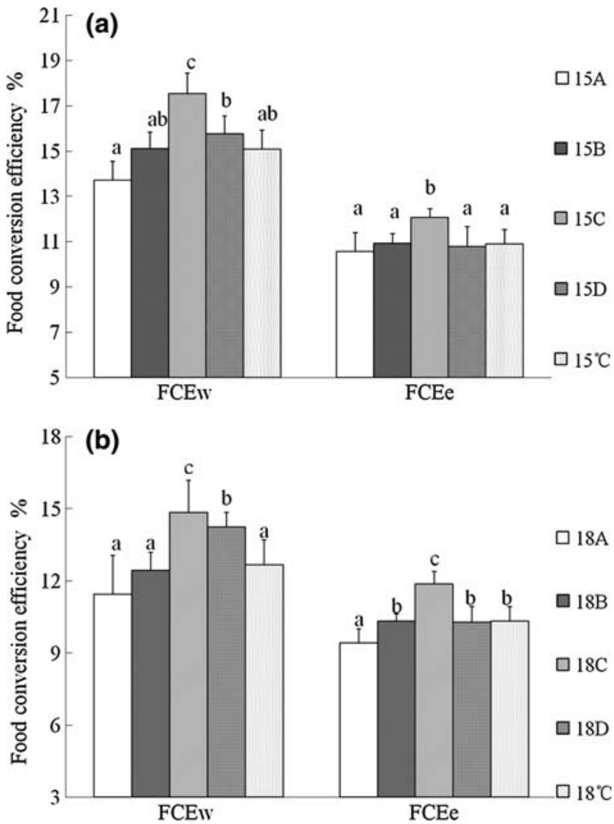

Fig. 3 The effects of patterns of diel fluctuating temperature on the FCE_w and FCE_e of the sea cucumber *Apostichopus japonicus* in the temperature regimes (**a**) 15 ± 3°C and (**b**) 18 ± 3°C. Histograms sharing a common letter are not significantly different ($P > 0.05$)

was significantly lower than that in treatments 15A and 15D ($P < 0.01$). Except for sea cucumbers in treatment 15B, the ratio R/C for sea cucumbers reared at fluctuating temperatures was significantly lower than that for sea cucumbers reared at constant temperature (15°C) ($P < 0.05$).

At a mean temperature of 18°C, the ratio G/C in treatment 18C was significantly higher than those in the other treatments ($P < 0.05$). R/C ratios in treatments 18A, 18C, and 18D were significantly lower than that for the constant temperature of 18°C ($P < 0.05$). The ratio F/C in treatment 18C was significantly lower than that in 18A and 18D ($P < 0.05$).

Discussion

Fluctuating temperatures can significantly affect the growth of the young sea cucumber *A. japonicus*, depending on the mean temperature and the amplitude of temperature fluctuation (Dong et al. 2006). Compared with the corresponding constant temperatures, growth rate increased for fluctuating temperatures with the mean temperature below the constant temperature optimum for growth (18°C) and decreased for fluctuating temperatures when the mean temperature was above 18°C.

Table 1 Energy budget for young *Apostichopus japonicus* for different modes of temperature fluctuation with mean temperatures 15 or 18°C and constant temperature of 15 or 18°C

Treatment	*G*/*C*[a]	*F*/*C*[b]	*U*/*C*[c]	*R*/*C*[d]
15A	33.83 ± 3.43^{ab}	26.48 ± 1.97^{b}	7.36 ± 0.54^{ab}	32.44 ± 3.49^{a}
15B	35.60 ± 3.06^{b}	24.34 ± 1.24^{ab}	6.71 ± 1.28^{ab}	33.41 ± 2.28^{ab}
15C	39.35 ± 2.58^{c}	22.97 ± 0.76^{a}	6.21 ± 1.25^{a}	31.44 ± 1.10^{a}
15D	34.23 ± 1.81^{ab}	26.67 ± 2.15^{b}	7.83 ± 0.51^{b}	31.44 ± 2.72^{a}
15°C	31.48 ± 2.67^{a}	24.11 ± 1.95^{a}	7.24 ± 1.29^{ab}	36.24 ± 2.04^{b}
18A	30.80 ± 1.52^{a}	26.00 ± 2.39^{b}	7.43 ± 0.33^{a}	35.82 ± 2.07^{a}
18B	30.98 ± 1.04^{a}	24.63 ± 1.72^{ab}	6.48 ± 1.49^{a}	37.91 ± 1.70^{ab}
18C	34.44 ± 1.61^{b}	22.58 ± 1.14^{a}	6.26 ± 0.48^{a}	36.76 ± 1.64^{a}
18D	30.90 ± 3.22^{a}	25.32 ± 2.06^{b}	7.44 ± 0.71^{a}	36.37 ± 1.40^{a}
18°C	30.99 ± 1.74^{a}	23.54 ± 1.05^{ab}	6.13 ± 1.57^{a}	39.32 ± 1.53^{b}

Values (expressed as mean ± SE, $n = 5$) with different lower case letters in the same column are significantly different from each other ($P < 0.05$)

[a] *G*/*C* (%) = (Energy for growth)/(energy consumed in food)

[b] *F*/*C* (%) = (Energy for feces)/(energy consumed in food)

[c] *U*/*C* (%) = (Energy for excretion)/(energy consumed in food)

[d] *R*/*C* (%) = (Energy for respiration)/(energy consumed in food)

To set up a more favorable temperature-fluctuation scheme, it is necessary to investigate the effect of mode of temperature fluctuation on growth of the sea cucumber *A. japonicus*. In this experiment, the modes of temperature fluctuation could affect growth of juvenile *A. japonicus*. Among the four modes of temperature fluctuation, the growth rate of sea cucumber in mode C was significantly higher than that for the corresponding constant temperatures. The other three modes, however, had no significant effect on growth of the sea cucumber. As reported after a previous study (Wei et al. 2004) and our observation (Meng and Dong unpublished data), the minimum and maximum values of water temperature in Jiaozhou Bay (120°07′ E–120°27′ E, 35°52′ N–36°13′ N) occurred at 0000 and 1400, respectively. The temperature-change mode is similar to that of mode C. Therefore, the higher growth rate of young sea cucumbers for mode C may be a evolutionary adaptation to natural seawater temperature.

Previous studies attributed the increase in growth under fluctuating temperature conditions to enhanced food intake (Diana 1984), reduced metabolism (Tian et al. 2004), and a change in the pattern of energy allocation (Cox and Coutant 1981; Konstantinov et al. 1989; Pilditch and Grant 1999). In this study, the values of FCE in mode C were significantly higher than for the other modes and the corresponding constant temperatures. This result indicates that sea cucumbers reared under optimum temperature-fluctuation conditions can utilize feed with greater efficiency. The data for energy allocation showed that sea cucumbers allocated more energy to growth and less energy to respiration when the mode of temperature fluctuation was optimum (Table 1). Energy allocated to feces was also different for different modes of temperature fluctuation, and the energy allocated to feces for sea cucumbers reared in mode C was significantly lower than that for the sea cucumbers reared in modes A and D.

Use of cDNA microarray analysis to examine changes in gene expression reveals that fluctuating temperature can affect cell growth and proliferation (Podrabsky and Somero 2004). At an appropriate fluctuation temperature, possible greater utilization of carbohydrate in the glycolytic cycle was found in our previous work (Dong et al. 2008), and for the large temperature fluctuation a higher level of Hsp70 indicated a possible high level of protein damage which might result in increased energy cost that could retard growth of sea cucumbers. In this study, different modes of temperature fluctuation might have different effects on gene expression and protein synthesis. More detailed studies are necessary to elucidate the biochemical and molecular mechanisms which affect the growth of the sea cucumber at fluctuating temperatures.

In conclusion, a temperature-control mode designed according to mode C, which simulates the natural fluctuation of the temperature of seawater, could significantly accelerate growth of juvenile sea cucumber *A. japonicus*. Growth rate was enhanced because less energy was allocated to respiration and feces. In culture of the sea cucumber, the temperature-control scheme designated mode C can enhance not only growth, but also efficiency of food utilization by the young sea cucumber.

Acknowledgments We thank Dr F. Wang for her technical support. We are grateful to X.W. Ren, F. Liu and C.Y. Ma for their help. The National Natural Science Foundation of China (grant no. 30400333, 30771661) supported this study.

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