Temperature effects on growth-ration relationships of juvenile sea cucumber *Apostichopus japonicus* (Selenka)

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Abstract

We studied the effects of temperature (16, 18, 20 and 22 °C) and ration (0%, 0.3%, 0.6% and 1.4% body weight day\(^{-1}\)) on the growth of juvenile sea cucumber *Apostichopus japonicus* (mean body weight 5.4±0.1 g) over 35 days. For any given ration, food intake (FI), specific growth rate (SGR) and food conversion efficiency (FCE) decreased with increasing temperature. The relationship among SGR\(_w\) (in terms of weight), SGR\(_e\) (in terms of energy), temperature (T), and ration (% body weight) could be described by the regression equations: SGR\(_w\)=0.132−0.024 T+0.284 R (\(r^2=0.724, n=64\)), and SGR\(_e\)=−0.014−0.030 T+0.312 R (\(r^2=0.654, n=64\)). These equations show that the minimum and maximum amount of energy required for maintenance occurred at 16 °C and at 22 °C, respectively. Furthermore, the energy requirements for maintenance at high temperatures exceeded the energy intake. The patterns of energy allocation revealed that energy required for respiration increased with increasing temperature. The aestivation of sea cucumbers in summer might be in response to a shortfall in energy intake with respect to an increase of energy consumed in respiration at high temperature.

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**Keywords:** Temperature; Ration; *Apostichopus japonicus*; Growth; Energy budget

1. Introduction

The sea cucumber *Apostichopus japonicus* Selenka, a holothurian believed to have aphrodisiac and medical properties, is becoming an important aquaculture species in China and Japan (Chen, 2004). Temperature has a major influence on growth and physiology (Li et al., 2002; Yang et al., 2005, 2006; Dong and Dong, 2006; Dong et al., 2006); and the optimum temperature for the growth of juvenile *A. japonicus* is about 15.5 °C (Dong et al., 2005). *A. japonicus* cease feeding and aestivate at temperatures of 24 °C and above (Liu et al., 1996; Yu and Song, 1999; Yang et al., 2005, 2006). Oxygen consumption of mature *A. japonicus* decreased when temperature exceeded the aestivation threshold, which depends on location, body size and age (Yang et al., 2005; Wang and Liao, 2001). The present study examined the effect of super-optimal temperature (16–22 °C) on the growth-food availability (ration) relations of juvenile *A. japonicus* to provide a preview for the large-scale sea cucumber aquaculture.

2. Materials and methods

2.1. Source of animals

The experiment was conducted from May 1 to July 5 2005 at the Mariculture Research Laboratory, Ocean University of...
China, Qingdao, P.R. China. The sea cucumbers were grown in and supplied by the ZhuoYue Aquaculture Corporation, Qingdao, P. R. China.

2.2. Acclimation and rearing conditions

Juvenile A. japonicus underwent a 10-day acclimation at 16 °C in continuously aerated seawater in several fiberglass tanks. At the end of this period, 192 animals of similar size (53.9 ± 0.16 g) were selected and transferred into 64 glass aquaria (450 mm × 250 mm × 350 mm) containing 30L seawater at different temperatures (16, 18, 20 and 22 °C) for a 7-day acclimation. Seawater used in the experiment was filtered using a sand filter. During the course of the experiment, water salinity was 28 ppt to 30 ppt. Water pH was around 7.8 and ammonia was less than 0.24 mg L⁻¹. Water salinity, pH and ammonia were determined with salinity refractometer (AIAGO, Japan), pH meter (PH3150i, WTW, Germany), and Hypobromite methods, respectively.

A simulated natural photoperiod (14-h light/10-h dark) was used. Each tank had a 1500W electric heater controlled via a thermostat. Aeration was provided continuously and the dissolved oxygen was maintained above 4.0 mg L⁻¹. During the acclimation period, the animals were fed ad libitum twice a day (0800 h and 1800 h) with sifted pieces of semi-dried Sargassum thunbergii (11.70% crude protein, 0.67% crude lipid, 12.89% ash, moisture < 1.00%; energy 8.55 kJ g⁻¹ dry mass) and the pieces (radius around 0.8 mm, weight around 2.5 mg) were insoluble in the sea water.

2.3. Experimental design and procedure

The acclimation was followed by starvation for 24 h. The initial individual wet weight measurements were taken with 1 min of removal from seawater and external water was removed from the specimens by drying them on sterile gauzes, then 64 individuals of similar weight (5.36 ± 0.03 g) were allocated to 64 aquaria (2 individuals / aquarium). There were 16 tanks (450 mm × 250 mm × 350 mm) at each of four temperatures (16, 18, 20 and 22 °C) with four tanks for each of the four levels of food supply (ration) (0%, 0.3%, 0.6% and 1.4% body weight day⁻¹). There was no significant difference in the initial body weight among the different treatments. The rearing conditions and feed were similar to those used during the acclimation period.

During the 35-day course of the experiment, the weight of each ration was recorded. Uneaten food and feces were removed by siphon and separated immediately to avoid decomposition, and then they were dried at 65 °C, ground, weighed and kept at −20 °C for the analysis of energy and nitrogen content. At the end of the experiment, the animals were starved for 24 h then weighed and dried at 65 °C for 48 h, and then ground to powders with a small grinder for measurement of energy and nitrogen content.

2.4. Energy determination and estimation of energy budget

The energy contents of the food, sea cucumber bodies, feces were measured with a Parr 1281 Oxygen Bomb Calorimeter (Parr, USA). The energy budget was calculated by the following equation (Carfoot, 1987):

\[ C = G + F + U + R \]

Where \( C \) is the energy consumed in food; \( G \) is the energy deposited as growth; \( F \) is the energy lost in feces; \( U \) is the energy lost in excretion and \( R \) is the energy used for respiration. The value of \( C \) and \( F \) can be calculated by the weight of the samples of food intake, feces weight and their energy content per gram. \( G \) can be calculated by the following equation:

\[ G = (F_w \times E_i) - (I_w \times E_i) \]

Where \( F_w \) and \( I_w \) are final body weight and initial body weight of the sea cucumbers, respectively; \( E_i \) and \( E_f \) are the energy content per gram of final body and initial body of the sea cucumbers, respectively.

The nitrogen contents of the sea cucumber bodies, food and feces were measured with a VarioEL III elemental analyzer (Elementar, Germany). The 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 from food; \( F_N \) is the nitrogen lost in feces; \( G_N \) is the nitrogen deposited in the body; 24,830 is the energy content (J g⁻¹) of excreted nitrogen.

The value of \( R \) was calculated by the energy budget equation:

\[ R = C - G - F - U \]

2.5. Calculation and data analysis

Specific growth rate in terms of weight (SGRw) and energy (SGR_e), food intake (FI), food conversion efficiency (FCE) and ration level (RL) were calculated as:

\[ SGR_w(\%day^{-1}) = 100 \times (\ln W_2 - \ln W_1)/D \]

\[ SGR_e(\%day^{-1}) = 100 \times (\ln E_2 - \ln E_1)/D \]

\[ FI(\%body weight day^{-1}) = 100 \times I/[D \times (W_2 + W_1)/2] \]

\[ FCE(\%) = 100 \times (W_2 - W_1)/I \]

\[ RL_w = 100 \times C_w/W_1 \]

\[ RL_e = 100 \times C_e/E_i \]
where, \( W_2 \) and \( W_1 \) are the final and initial body weight of the sea cucumbers, respectively; \( E_2 \) and \( E_1 \) are the final and initial energy content of the sea cucumbers, respectively; \( D \) is the duration of the experiment; \( C_w \) and \( C_e \) are the total weight of food and energy consumed, respectively; \( I \) is the total food intake during the whole experiment. \( RL_w \) and \( RL_e \) are ration level in terms of weight and energy.

The data were analyzed by the SPSS for Windows (Version 10.0) statistical package. Two-way analysis of variance (ANOVA) was used to test for the effect of the interaction between temperature and ration on growth, FI and FCE. The effects of temperature and ration on SGR were tested using stepwise multiple regression analysis. Differences were considered statistically significant if \( P < 0.05 \).

### 3. Results

#### 3.1. Growth

The maximum and minimum of SGR (SGR\(_w\) and SGR\(_e\)) occurred at treatment (16 °C, 1.4% ration) and treatment (22 °C, starvation), respectively. Stepwise multiple regression analysis showed that SGR\(_w\) and SGR\(_e\) decreased with increasing temperature, and increased with increasing ration at any given temperature (Fig. 1). The relationship among SGR\(_w\) and SGR\(_e\), temperature (T) and ration (Ra) can be described by the regression equations:

\[
\text{SGR}_w = 0.132 - 0.024T + 0.284Ra (r^2 = 0.724, n = 64) \tag{1}
\]

\[
\text{SGR}_e = -0.014 - 0.030T + 0.312Ra (r^2 = 0.654, n = 64) \tag{2}
\]

The results of the two-way ANOVA analysis showed no significant interaction between the temperature and the ration on SGR of \( A. \) japonicus \( (P > 0.05) \). According to the equation, growth decreased with increasing temperature beyond the optimum, and increased with increasing ration.

According to the Eq. (1), the maintenance ration levels in terms of weight (RL\(_w\)) at 16 °C, 18 °C, 20 °C and 22 °C were 0.91%, 1.08%, 1.25% and 1.43% of body weight, respectively. According to the Eq. (2) the maintenance ration levels in terms of energy at 16 °C, 18 °C, 20 °C and 22 °C were 1.59%, 1.78%, 1.98% and 2.17% of body energy, respectively. The minimum and maximum maintenance rations occurred at 16 °C and 22 °C, respectively. The maintenance ration levels increased with increasing temperature.

#### 3.2. Food intake (FI), food conversion efficiency (FCE)

FCE decreased with increasing temperature \( (P < 0.05) \). There was no significant difference in FI between different temperatures \( (P > 0.05) \) (Table 1). FCE and FI were both affected significantly by ration \( (P < 0.05) \). The highest FCE occurred at treatment (16 °C, 1.4% ration). FCE and FI were not significantly affected by the interaction of temperature and ration \( (P > 0.05) \).

#### 3.3. Energy allocation

The patterns of energy allocation in the test animals revealed significant differences among treatments (Table 2). In the temperature range of this experiment, the energy deposited as growth decreased with rising temperature \( (P<0.05) \). The consumption of energy for respiration increased with increasing temperature \( (P<0.05) \). Temperature had no significant influence on fecal or excretion energy \( (P>0.05) \).

### 4. Discussion

Although temperature is an important factor that affects the growth and metabolism of \( A. \) japonicus, there is still limited information available, especially about growth at temperatures below the threshold for aestivation (Dong...
Table 1
Effect of temperature and ration on the feed intake (FI) and food conversion efficiency (FCE) in sea cucumber *A. japonicus*. 

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Ration (%)</th>
<th>FCE (% day⁻¹)²</th>
<th>FI (% day⁻¹)³</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>0.3</td>
<td>-121.4±15.75</td>
<td>0.26±0.01</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>5.57±0.84</td>
<td>0.50±0.02</td>
</tr>
<tr>
<td></td>
<td>1.4 (Satiation)</td>
<td>9.22±2.16</td>
<td>1.13±0.05</td>
</tr>
<tr>
<td>18</td>
<td>0.3</td>
<td>-102.31±10.26</td>
<td>0.26±0.08</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>4.35±3.04</td>
<td>0.52±0.01</td>
</tr>
<tr>
<td></td>
<td>1.4 (Satiation)</td>
<td>7.25±2.57</td>
<td>1.08±0.04</td>
</tr>
<tr>
<td>20</td>
<td>0.3</td>
<td>-114.43±5.03</td>
<td>0.25±0.01</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>-1.32±0.76</td>
<td>0.46±0.03</td>
</tr>
<tr>
<td></td>
<td>1.4 (Satiation)</td>
<td>1.49±0.52</td>
<td>1.14±0.10</td>
</tr>
<tr>
<td>22</td>
<td>0.3</td>
<td>-133.61±8.67</td>
<td>0.25±0.01</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>-31.53±9.39</td>
<td>0.51±0.02</td>
</tr>
<tr>
<td></td>
<td>1.4 (Satiation)</td>
<td>-9.49±2.11</td>
<td>1.11±0.04</td>
</tr>
</tbody>
</table>

¹Values (mean±S.E. of four replications) in the same column not sharing a common superscript letter are significantly different (P<0.05).
²FI (%)=100×D/(W2−W1)/2.
³FCE (%)=100×(W2−W1)/F.

Table 2
Energy budgets in *A. japonicus* at different temperatures and rations. 

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Ration (% body weight day⁻¹)</th>
<th>Food consumption (J.g⁻¹.d⁻¹)</th>
<th>Growth (J.g⁻¹.d⁻¹)</th>
<th>Faecal (J.g⁻¹.d⁻¹)</th>
<th>Excretion (J.g⁻¹.d⁻¹)</th>
<th>Metabolism (J.g⁻¹.d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16 °C</td>
<td>0.00</td>
<td>-233.98±26.63</td>
<td>37.33±1.43</td>
<td>152.62±6.26</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.30</td>
<td>-233.98±26.62</td>
<td>36.65±1.77</td>
<td>169.02±11.21</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.60</td>
<td>116.86±7.85</td>
<td>60.7±6.47</td>
<td>181.74±19.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>242.58±13.65</td>
<td>79.20±3.46</td>
<td>254.04±14.54</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.4 (Satiation)</td>
<td>540.07±24.82</td>
<td>120.77±26.37</td>
<td>177.93±23.45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 °C</td>
<td>0.00</td>
<td>-233.98±26.62</td>
<td>36.65±1.77</td>
<td>181.74±19.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.30</td>
<td>-117.56±1.39</td>
<td>47.59±3.75</td>
<td>195.21±24.77</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.60</td>
<td>243.08±12.06</td>
<td>71.48±3.13</td>
<td>255.71±17.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.4 (Satiation)</td>
<td>505.69±49.98</td>
<td>140.7±25.24</td>
<td>323.93±70.37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 °C</td>
<td>0.00</td>
<td>-248.56±17.95</td>
<td>38.90±2.69</td>
<td>209.66±15.84</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.30</td>
<td>-111.32±7.52</td>
<td>44.76±3.52</td>
<td>239.95±26.65</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.60</td>
<td>218.53±20.99</td>
<td>64.51±2.82</td>
<td>313.39±15.62</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.4 (Satiation)</td>
<td>543.65±51.58</td>
<td>139.99±15.75</td>
<td>367.87±9.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22 °C</td>
<td>0.00</td>
<td>-284.27±47.43</td>
<td>53.85±4.48</td>
<td>230.41±43.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.30</td>
<td>-108.54±4.46</td>
<td>39.58±3.11</td>
<td>272.86±11.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.60</td>
<td>237.74±14.82</td>
<td>59.45±2.60</td>
<td>314.17±21.97</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.4 (Satiation)</td>
<td>504.52±24.78</td>
<td>64.45±25.00</td>
<td>420.56±46.96</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹Common letter denotes no significant difference between means (P<0.05).
decrease of FCE and the increase of energy consumed by respiration. Due to the low energy content of its food (mainly contains alga pieces, mud and organic debris) and its low eating efficiency, sea cucumber *Apostichopus japonicus* cannot obtain plentiful energy everyday. At the temperature beyond 22 °C (near the temperature threshold for aestivation), energy requirement exceeded energy intake. Therefore, sea cucumber *Apostichopus japonicus* has to decrease its energy requirement and metabolic rate by aestivation when ambient temperature exceeds the threshold point.

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