Feeding characteristics of juvenile Japanese sea cucumber *Apostichopus japonicus* (Stichopodidae) in a nursery culture tank

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Abstract : The cause of differences in individual growth rates (uneven growth) of Japanese sea cucumber *Apostichopus japonicus* seedlings was investigated by changing the conditions of rearing density and food amount in a nursery culture experiment. As a result, when the animals were fed, some of the animals grew rapidly so uneven growth occurred regardless of the density. In other animals, the growth rate was closer to that of the non feeding condition, and it was suggested that the uneven growth might occur under the condition of the occurrence of some animals being satiated and others starving. Further, assuming such an unevenness of food amount was derived from the unevenness of opportunity to eat, the feeding method was improved, and the uneven growth was kept smaller than before (usual feeding method). Therefore, unevenness of the available food amount was considered to be the cause of the uneven growth of the sea cucumber seedlings.

Key words : Benthos, Echinoderm fisheries, Ecology, Feeding, Growth

Introduction

The Japanese common sea cucumber *Apostichopus japonicus* (Selenka, 1867) is distributed widely in shallow coastal areas in the Northwest Pacific, and throughout mainland Japan from Hokkaido to Kagoshima.[1] It is a commercially important species and its stock-enhancement program is conducted. In Japan, the technique of artificial juvenile production was established for release of these seedlings,[2] and reached a stage of mass production in recent years. However, in the next step of development, nursery culture for the production of large sized seedlings, it becomes a serious problem that seedlings show pronounced differences in the individual growth rate (this is called "uneven growth" hereinafter).[2,3] Hatanaka[4] reported that the uneven growth of seedlings was enhanced by over-

crowding, and suggested that overcrowding itself or ensuing competition among the individuals for food would be the factor leading to the uneven growth. That could also be enhanced by another reason: individual difference in the available food amount, suggested by the observation that many of the seedlings attached to the wall of the rearing tank could not eat the food scattering on the bottom of the tank.[5] However, presently, the cause (direct or indirect) of the uneven growth is still not clear. In general, under the condition of uneven growth, very few individuals grow rapidly, and the others grow slowly with some individuals showing negative growth. Therefore, the nursery culture results in very low production efficiency. For the same reason, since it is difficult to determine the growth rate of experimental animals and compare the growth among the groups, we cannot set new subject for development of effec-

tive food and the method of rearing. In the present study, in order to clarify the cause of the uneven growth of *A. japonicus*, we performed a nursery culture experiment using simulated culture tanks (test tanks) on a small scale, and examined the effect of the rearing method on the growth of seedlings.

Materials and methods

Two experiments were performed in this study by rearing artificial seedlings of *A. japonicus* (green color type) kept in test tanks and measured periodically. Since it is suggested that overcrowding and starving caused the uneven growth,[4] first, we conducted experiments under several conditions of rearing densities and food amounts, and investigated the cause of the uneven growth by comparing the conditions that showed the smallest deviation of body size with those showing the largest (experiment I). Second, we investigated if it could be realized or not that uneven growth was maintained at a deceleration by the addition of a special technique to the feeding method (experiment II). Both sets of seedlings for experiment I and II were produced by the Sasebo City Suisan-Center, in April 2004 and 2005 respectively, and fed on diatom, *Chaetoceros*, and seaweed powder (Rivic BW, Riken Vitamin Co., Ltd., Tokyo, Japan).

Experiment I (factors causing the uneven growth)

All animals were transported to the Tana Marine Biological Laboratory (TMBL) of National Fisheries University in August 2004, and reared for 176 days from 19 August to 10 February 2005 after the acclimation to the rearing environment. The experiment was carried out in 8 rearing tanks, PVC rectangular tanks with a base 60 cm×30 cm and a height of 20 cm, and with water depth 15 cm (Fig. 1). The experimental conditions were defined by the food amount (feeding or without feeding) and initial density (5 or 30 individuals in a tank). Animals were allocated randomly in the tanks in the following four conditions: feeding – low density (F-5); non feeding – low density (N-5); feeding – high density (F-30); non feeding – high density (N-30). At the start of the rearing, animals varied within body length of 5-25 mm by visual assessment without anesthesia.[6] Then, for the purpose of arranging animals of approximately the same size in each tank, animals were divided into two size groups as follows : small sized animals within body length of 5-15 mm by visual assessment (F-5 -s, N-5-s, F-30-s, N-30-s); large sized animals of 15-25 mm (F-5-l, N-5-l, F-30-l, N-30-l).

Animals accommodated in a tank under the feeding condition (F-5-s, F-30-s, F-5-l, F-30-l) were fed Rivic BW at the amount of 5 g per a tank, three times a week at
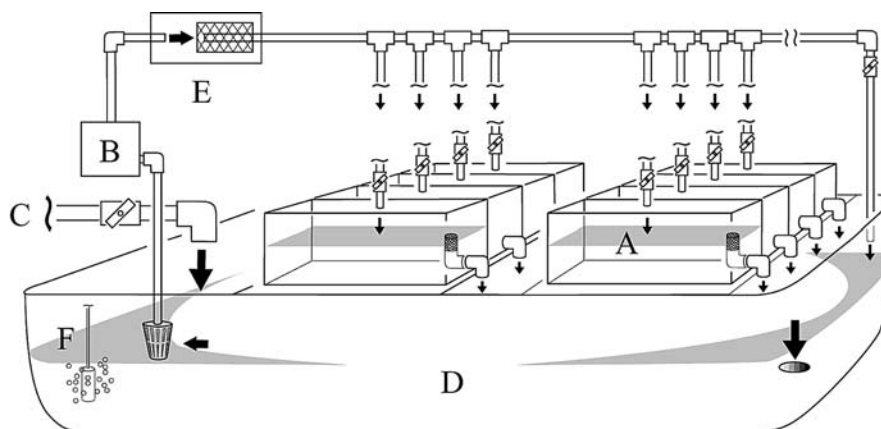

Fig. 1. Diagram of the rearing tank for experiment I (factors causing the uneven growth of *Apostichopus japonicus*). Arrows indicate water flow.
A : PVC rearing tank (60×30×20 cm) (grey colored)
B : motor-driven water pump
C : sand filtered seawater
D : FRP sedimentation tank (476×145×90 cm)
E : PVC sedimentation tank (60×50×30 cm) with filter mesh (size of 500×500 μm)
F : aerator

8:00‐9:00 A.M. and animals without feeding (N‐5‐s, N‐30‐s, N‐5‐l, N‐30‐l) were not fed at all. In general, the non feeding treatment does not cause death by starvation in this species but may lead to reduced size. Rivic BW was given over the whole surface of the bottom of the tank, in a form of mashed mixture with seawater. This experiment was continued without cleaning for the residual feed and feces in the tank. Inside of the tanks, there was no sand or any kind of sheltering such as stones, which is the usual procedure for *A. japonicus* rearing. To prevent algal growth as a result of direct sunlight, the experimental tanks were placed indoors, where the luminous intensity was less than 500 lux even in the daytime. Rearing seawater was pumped from Hirao Bay in front of TMBL, where *A. japonicus* is abundant, and was supplied to the experimental tanks after the sand filtration and ventilation. The water flow was at a rate of approximately 0.5 liter per minute in each rearing tank. No heating or cooling equipment was used. All animals were measured for body length in mm under anesthesia,[6] on 18 August 2004, 4 November, and 10 February 2005; approximately three and six months after the start of rearing, respectively.

Experiment II (reduction of the uneven growth)

All animals were transported to the National Fisheries University (NFU) in July 2005, and reared for 242 days from 28 July to 26 March 2006, after they had been acclimated to the rearing environment. PVC rectangular tank with a base of 40 cm×20 cm and a height of 30 cm was used, with water depth 25 cm (Fig. 2). Only large sized animals within body length of 15‐25 mm by visual assessment were used for the experiment. In this study, two kinds of feeding method, new method and usual method, were used. The new method enabled the animals to take in feed on the wall of rearing tank (whole surface feeding). On the other hand, the usual method feed is given on the bottom of the tank, in the same method of feeding of experiment I (bottom surface feeding). The experiment was conducted under two conditions of initial density (5 or 50 individuals in a tank), which could be expected to affect uneven growth.[4] Animals were accommodated randomly in a separate tank in the following four conditions : whole surface feeding‐low density (W‐5); bottom surface feeding‐low density (B‐5); whole surface feeding‐high density (W‐50); bottom surface feeding‐high density (B‐50).

The whole surface feeding method was to apply a thin coat of mashed mixture of food to the wall of the tank by using a paint brush. The bottom was excluded from this operation in anticipation of settlement of suspended food particles in the water. For the purpose of making the feed particles easier to be attached to the surface of the wall, all

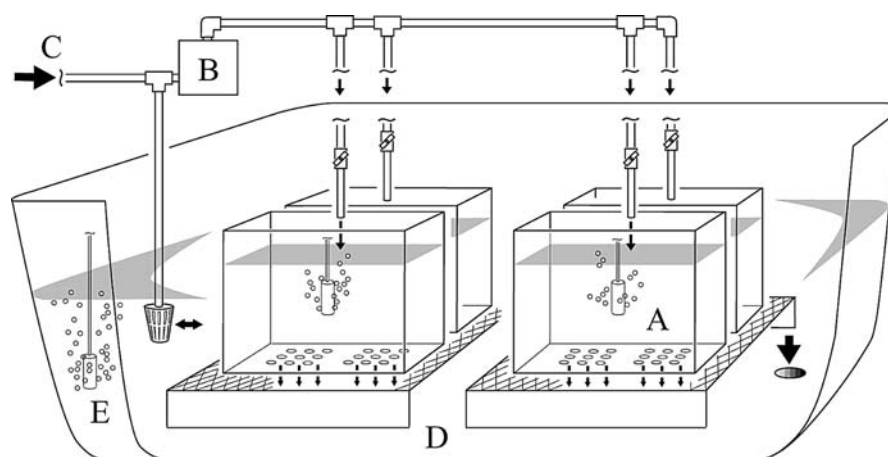**Fig. 2 .** Diagram of rearing tank for the experiment II (reduction of the uneven growth of *Apostichopus japonicus*). Arrows indicate water flow.
A : PVC rearing tank (40×20×30 cm) (white colored)
B : motor‐driven water pump
C : sand filtered seawater
D : FRP sedimentation tank (200×100×100 cm)
E : aerator

surfaces of the wall were covered with nylon mesh (white colored mesh, size 1×1 mm).

Froze (-40℃) raw kelp *Undaria pinnatifida* (Phaeophyceae) was used as food, after the homogenization by grinding to particles of approximately 1×1 mm using motor-driven juicer. Before the feeding, the mashed kelp was added to the food powder, which consisted of $2:1:4:4$ mixture of Rivic BW, de-fatted soybean powder (Nippon Nosan Kogyo Co., Ltd., Kanagawa, Japan), material powder of mixed food for abalone (Awabi, Nippon Nosan Kogyo Co., Ltd., Kanagawa, Japan), and mixed food powder for young carp (Koi-M, Hayashikane Sangyo Co., Ltd., Yamaguchi, Japan) at portions of five percent (w/w). Feeding was performed once a day at 6:00-7:00 P.M. Daily food amount was increased following the increase in body size as follows : 25 g on July-October, 50 g on November-January, and 75 g on February-March. In this study, 75 g was the limit of capacity of the whole surface feeding with no remaining capacity of the mesh for the feed brushing.

The foods of both feeding method were the same and the amounts were also equal. The bottom surface feeding method was to gently pour the mashed mixture of food uniformly onto the bottom of tank. To prevent the food particles from attaching to the wall, nylon mesh was not used. To remove the remainder of food, feces and dead animals, animals of both feeding conditions were taken out from their tank before feeding, and all tanks were washed with a hard brush. Animals were returned to their tank after the feed had been given. In the same manner as experiment I, neither stone nor sand was set on the bottom, and tanks were placed indoors. Luminous intensity was less than 200 lux even in the daytime. The layout of rearing tanks was rearranged randomly every week. Rearing seawater was pumped up from the Sea of Japan adjacent to NFU, where *A. japonicus* is abundant, and was supplied to the experimental tanks after the sand filtration and ventilation. The water flow was at a rate of approximately 0.5 liter per minute in each rearing tank. No heating or cooling equipment was used. Because of the possibility of oxygen deficiency in the high density tanks, inside of all rearing tanks was provided with aeration, which was controlled moderately so as not to agitate the food particles.

All animals were removed for size measurement every month. Photographs of each group was taken in a white aquaria, and animals were measured body length (L) and breadth (B) to the nearest mm from the pictures for calculating the estimates of the anesthetized body length (Le).[7] Here, Le is the size criterion of *A. japonicus*, given in the formula [Le (mm) $= 2.32 + 2.02 \times (L \times B)^{1/2}$] for the green color type of *A. japonicus*, and can be obtained from the animals extending and contracting freely without anesthesia. At the end of the experiment, estimates of the wet weight [W(g) $= 3.62 \times 10^{-5} \times Le^{2.98}$] were also calculated.[7] Also the anesthetized body length and anesthetized wet weight were measured to obtain the correction factor for these estimates, by using the anesthetizer designed for adult sea cucumber.[8] For anesthetizer, the standard liquid, 10% ethyl alcohol seawater-solution saturated with menthol, was prepared. Then, the standard liquid was diluted to 40% with seawater, and used as the anesthetizer immediately.[8] Animals were immersed in 0.5-1 liter of anesthetizer for 10-60 minutes. After the measurements, all animals were fixed with a 70% methanol solution, and dissected.

In addition, feeding motions and attachment positions of all animals in each tank were observed 25 times at intervals of one hour from midday on February 16 to midday February 17, 2006, to examine the differences of diurnal motions among the rearing conditions. In this study, we assumed feeding motions for all actions that animal moving its circumoral-tentacles. We classified the attachment positions into two groups, the wall and the bottom, which was further divided into subdivision as follows : the wall adjacent to the bottom and another, the bottom adjacent to the wall and another, respectively.

Results

Throughout all the experiments of this study, animals did not show any aggregation behavior for feeding. Usually, many animals attached to the walls of the tank, and a few attached to the bottom, regardless of the occurrence of food. Even on the bottom, most animals accumulated at sections of the bottom adjacent to the walls, and the animals feeding actions were mainly observed on the position where

many animals were attached. Therefore, on the bottom of the rearing tank, it was often observed that most of the food remained except the part adjacent to the walls.

Experiment Ⅰ (factors causing the uneven growth)

Water temperature in the rearing tank fluctuated between 4.4℃ and 27.5℃ during the experimental period. Six months after the start, survival rates of the animals under the feeding condition showed small percentages within 3-40% (av. 14%), resulting in stopping of the rearing (Table 1). At the start of the experiment, animals kept in each of the four tanks of small and large sized animals were identical in both variance and average of body size (variance: Bartlett test for homogeneity of variances, $Ps > 0.05$) (average: one-way ANOVA, $Ps > 0.05$). However, three months after the start, they were significantly different in both variance and average size (variance: Bartlett test for homogeneity of variances, $Ps < 0.05$) (average: Kruskal-Wallis test, $Ps < 0.05$) (Fig. 3, Table 1). The maximum values when the animals were kept at low density and fed (F-5-s, F-5-l), and the minimum values when kept at high density and without feeding (N-30-s, N-30-l), and the average of these two conditions were significantly different (Steel-Dwass multiple comparison, $Ps < 0.05$). When comparing body size at the start and three months after the start, variance was significantly increased when animals were fed (F-5-s, F-30-s, F-5-l, F-30-l) in both small and large sized animals (F-test, $Ps < 0.005$). As an additional remark, among these fed animals, all surviving animals in the low-density condition (F-5-s, F-5-l) increased in size, particularly the large sized animals (F-5-l) significantly increased even the average value (Cochran-Cox test, $P < 0.05$). On the other hand, in the high-density condition (F-30-s, F-30-l) at three months after the start, the small and large sized animals, that had larger values than the range of initial body size, were only two and three individuals (25% and 27% of survival animals), respectively. Furthermore, when these well-grown animals were removed, the variance of body size at three months after the start was not significantly different from that at the start (F-test, $Ps > 0.05$), and the average decreased in both small and large sized animals, especially in the large sized

Table 1 . Number surviving and size changes of reared animals in experiment I (factors causing the uneven growth of *Apostichopus japonicus*)

	Group*[1]	Aug. 18, '04	Nov. 4	Feb. 10, '05
Number surviving	F-5-s	5	3	1
	N-5-s	5	4	4
	F-30-s	30	8	1
	N-30-s	30	21	13
	F-5-l	5	5	2
	N-5-l	5	5	4
	F-30-l	30	11	6
	N-30-l	30	29	15
Range (mm) (av. mm)	F-5-s	11 - 15 (12.8)	20 - 42 (29.0)	56 -
	N-5-s	10 - 15 (12.4)	6 - 16 (11.5)	6 - 29 (14.3)
	F-30-s	7 - 19 (12.5)	9 - 41 (17.1)	78 -
	N-30-s	9 - 18 (12.3)	6 - 17 (10.3)	3 - 22 (12.8)
	F-5-l	18 - 24 (21.4)	26 - 58 (41.2)	28 , 37 -
	N-5-l	17 - 27 (21.0)	11 - 31 (21.6)	21 - 30 (26.0)
	F-30-l	14 - 28 (20.1)	9 - 50 (21.5)	31 - 88 (48.7)
	N-30-l	15 - 26 (20.4)	8 - 20 (13.7)	6 - 29 (12.7)
s.d. (mm) (c.v. %)	F-5-s	1.5 (11.6)	11.5 (39.8)	- -
	N-5-s	2.1 (16.7)	4.2 (36.5)	10.9 (76.5)
	F-30-s	3.1 (25.1)	10.5 (61.4)	- -
	N-30-s	2.7 (22.2)	3.0 (29.3)	9.5 (7.4)
	F-5-l	2.3 (10.8)	12.7 (30.8)	- -
	N-5-l	3.9 (18.8)	7.1 (32.8)	3.7 (14.3)
	F-30-l	3.7 (18.4)	13.5 (62.8)	22.1 (45.5)
	N-30-l	2.6 (12.9)	2.9 (21.4)	6.1 (47.9)

[1] F-5: feeding - low density; N-5: non-feeding - low density; F-30: feeding - high density; N-30: non-feeding - high density. "-s" and "-l" groups using small and large sized animals, respectively.

animals (F-30-l), and that was significantly different from at the start (t-test, $Ps < 0.05$). However, in the non feeding condition (N-5-s, N-30-s, N-5-l, N-30-l), body sizes at three months after the start were not significantly different
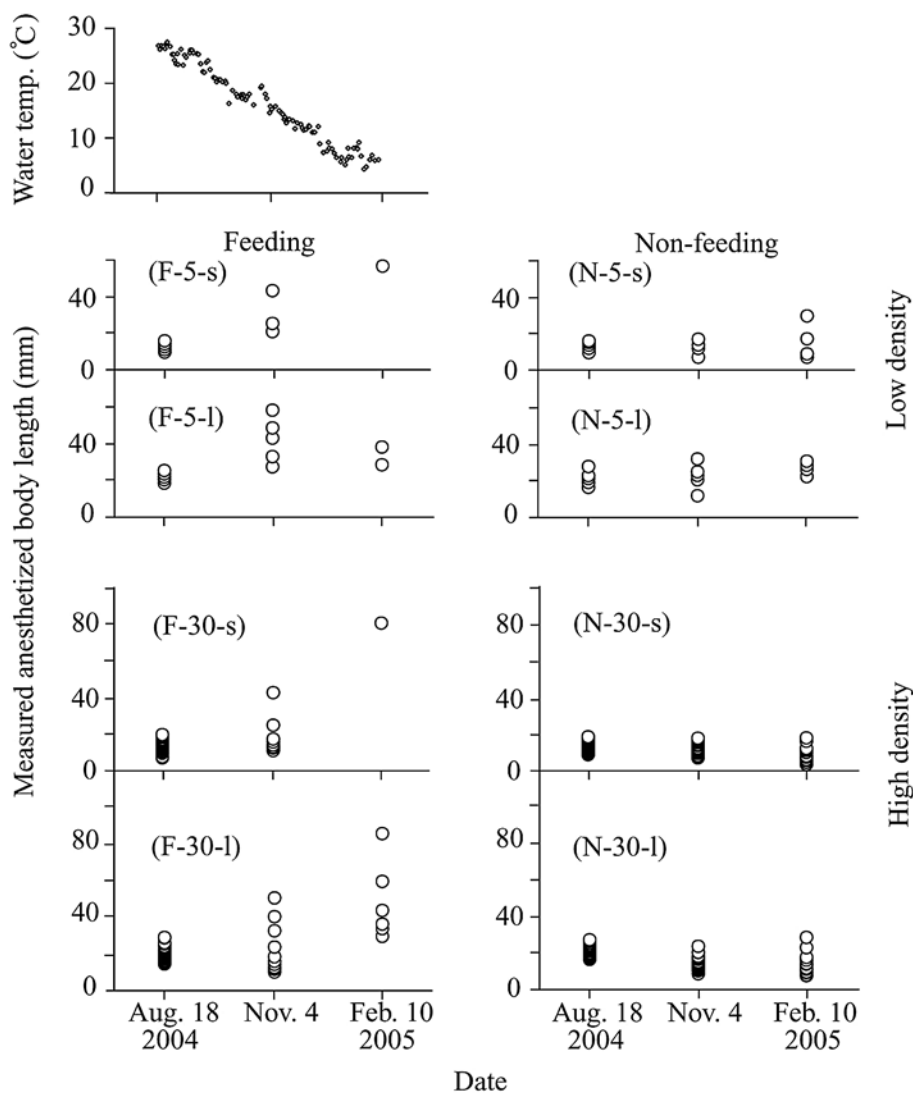
Fig. 3. Size changes of rearing animals in experiment I (factors causing the uneven growth of *Apostichopus japonicus*) and daily changes of water temprature in the rearing tank. Size measurements were performed every 3 months by using *l*-menthol anesthetizer.[6] Symbols F- and N- indicate the feeding condition (groups F-5-s, F-30-s, F-5-l, and F-30-l) and non feeding condition (groups N-5-s, N-30-s, N-5-l, and N-30-l). Low-density tanks (groups F-5-s, F-5-l, N-5-s, and N-5-l) and high-density tanks (groups F-30-s, F-30-l, N-30-s, and N-30-l) were initially stocked with 5 and 30 animals, respectively. The experimental groups that were composed of the small-sized animals (5-15 mm) and large-sized animals (15-25 mm) were indicated by "-s" and "-l", respectively.

from the initial body size in both variance and average (variance : F-test, *Ps* > 0.05) (average : t-test, *Ps* > 0.05).

Experiment II (reduction of the uneven growth)

Water temperature in the rearing tank fluctuated between 9.5℃ and 28.6℃ during the experimental period. At the start of the experiment, in the rearing tank for the whole surface feeding (W-5, W-50), many bubbles attached to the mesh on the walls, and animals, which attached to mesh with bubbles, were trapped by bubbles and their actively were dull. Then, bubbles disappeared within about one week after the start, and the animals recovered. In the high-density condition (W-50, B-50), many animals died followed by evisceration within one month from the start of rearing. During that high-mortality period, 34% and 26% of the animals died in the W-50 and B-50, respectively, and the average size of surviving animals de-

creased in both feeding conditions (Fig. 4, Table 2). The same order of high-mortality in a short period did not occur in the low-density condition (W-5, B-5). In this experiment, from the beginning to the end, the variance of body size was not significantly different among the tanks (F-test, $Ps > 0.05$). On average, animals were larger in the whole surface feeding (W-5, W-50) than in the bottom surface feeding (B-5, B-50) from three months after the start. And also, there were significant differences of the average in the low-density condition (W-5, B-5) and in the high-density condition (W-50, B-50) since seven and

five months after the start, respectively (t-test, $Ps < 0.05$). Then, we calculated the coefficient of variance, standard deviation divided by the average, for estimation of uneven growth. The coefficient of variance of the body length was smaller in the whole surface feeding (W-5, W-50) than in the bottom surface feeding (B-5, B-50) since one month after the start, regardless of the density condition (sign-test, $Ps < 0.05$) (Table 2). However, even in the whole surface feeding, some of the animals in the high-density condition (W-50) decreased in size at eight months after the start, and the coefficient of variance suddenly increased
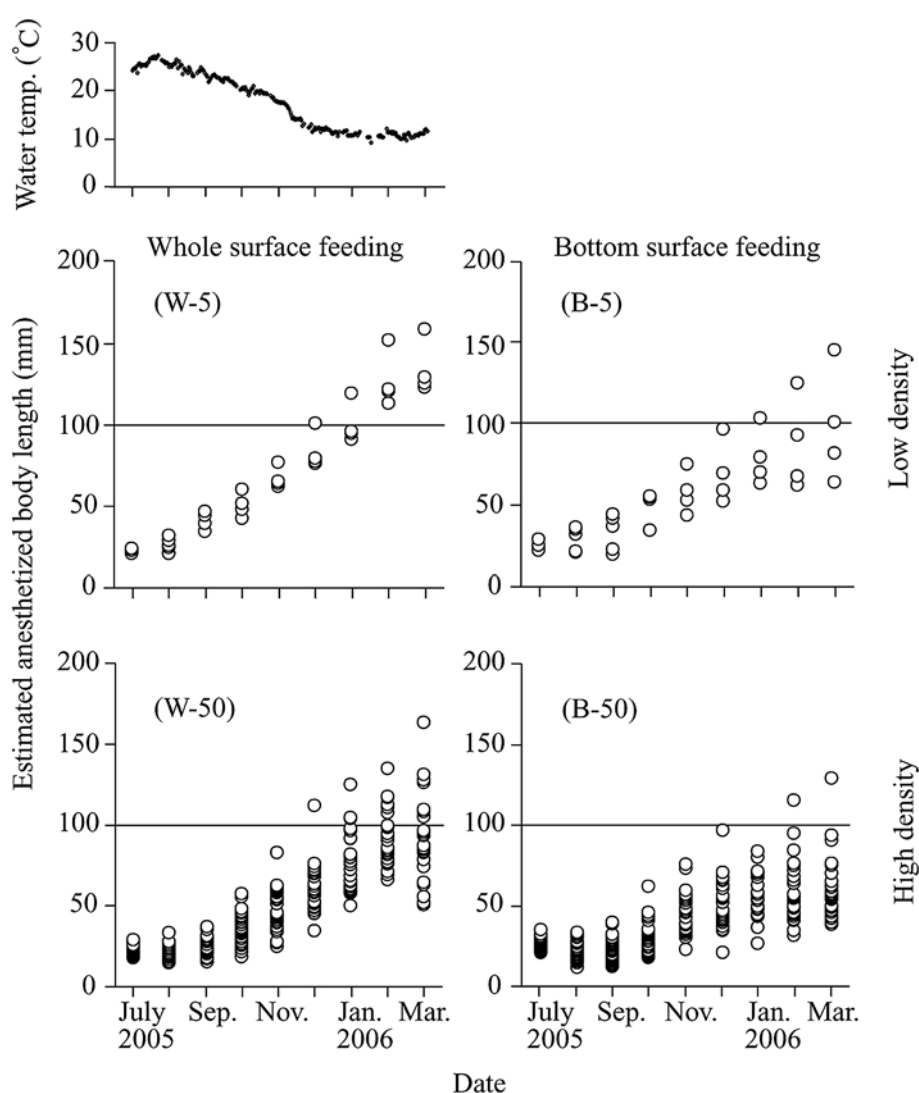

Fig. 4. Size changes of reared animals in experiment II (reduction of the uneven growth of *Apostichopus japonicus*) and daily changes of water temperature in the rearing tank. Body sizes were estimated every month by using regression equation,[7] that calculating anesthetized body length from one simultaneous measurement of the body length and the body breadth without anesthetizer. Symbols W-5, B-5, and W-50, B-50 indicate the experimental groups that were initially stocked with 5 animals (low-density) and 50 animals (high-density), respectively.

and approached that of the bottom surface feeding. When the average given each four well-grown animals were compared for the purpose of considering the number surviving in the low-density conditions (W-5, B-5), the ranking was as follow : W-5＝W-50＞B-5＞B-50 (Table 3).

In this study, during the 25 hourly observations of the animals feeding motions and attachment positions no difference was observed among the tanks (Fig. 5). The feeding motions followed a diurnal pattern, that animals were activate mainly in the nighttime from evening to morning, and were most frequent in the midnight. More than 40% of animals were considered to be showing feeding motions in all tanks at 0 A.M. Attachment positions did not follow a diurnal pattern. The frequencies of attachment positions were higher in the wall than the bottom common to all tanks. However, based on a comparison of surface areas, such a tendency was not seen for reason of the shape of tanks, that had larger area in the wall than in the bottom

Table 2 . Number surviving and size changes of rearing animals in experiment II (reduction of the uneven growth of *Apostichopus japonicus*)

	Group[2]	July 13, '05	Aug. 27	Sept. 28	Oct. 27	Nov. 29	Dec. 30	Jan. 27, '06	Feb. 26	Mar. 26
Number surviving	W-5	5	5	4	4	4	4	4	4	4
	B-5	5	5	5	4	4	4	4	4	4
	W-50	50	33	33	33	31	25	25	25	25
	B-50	50	37	37	31	31	27	26	26	25
Range (mm) (av. mm)	W-5	20 - 23 (21.8)	20 - 31 (25.8)	34 - 47 (40.7)	42 - 60 (50.2)	62 - 77 (66.7)	77 - 100 (83.2)	91 - 119 (100.1)	113 - 152 (126.7)	123 - 159 (134.0)
	B-5	21 - 28 (23.9)	20 - 36 (28.5)	19 - 44 (32.4)	34 - 55 (49.3)	43 - 74 (57.0)	52 - 96 (68.6)	62 - 103 (78.1)	61 - 124 (86.0)	63 - 144 (97.1)
	W-50	19 - 29 (23.0)	14 - 33 (21.0)	15 - 37 (24.4)	18 - 57 (35.3)	25 - 82 (47.5)	34 - 112 (60.4)	50 - 125 (76.6)	66 - 135 (91.1)	50 - 163 (91.6)
	B-50	18 - 34 (24.2)	10 - 33 (20.2)	12 - 39 (21.5)	17 - 61 (29.0)	23 - 75 (43.6)	22 - 97 (50.7)	26 - 83 (54.8)	31 - 115 (57.7)	39 - 128 (60.9)
s.d. (mm) (c.v. %)	W-5	1.2 (5.5)	4.4 (17.1)	5.5 (13.4)	7.4 (14.8)	6.8 (10.3)	11.5 (13.8)	13.0 (13.0)	17.0 (13.4)	16.6 (12.4)
	B-5	2.7 (11.3)	7.3 (25.8)	11.4 (35.0)	10.3 (20.9)	13.1 (23.1)	19.4 (28.2)	17.6 (22.5)	28.5 (33.1)	34.7 (35.7)
	W-50	2.1 (9.2)	4.0 (19.3)	5.4 (22.0)	9.2 (26.1)	12.8 (27.0)	15.0 (24.8)	18.6 (24.3)	17.5 (19.2)	26.6 (29.0)
	B-50	4.0 (16.4)	6.3 (31.3)	6.8 (31.6)	10.1 (34.7)	12.9 (29.5)	15.2 (30.1)	14.1 (25.6)	19.2 (33.4)	19.9 (32.7)

[2] W: whole surface feeding; B: bottom surface feeding. "-5" and "-50" groups have low and high density, respectively.

Table 3 . Measurements of the largest 4 individuals of each rearing tank at the end of experiment II (reduction of the uneven growth of *Apostichopus japonicus*)

		Body length				Wet weight			
		Estimated[4]		Measured[5]		Estimated[4]		Measured[5]	
Group[3]	Number surviving	Range (mm) (av. mm)	s.d. (mm) (c.v. %)	Range (mm) (av. mm)	s.d. (mm) (c.v. %)	Range (g) (av. g)	s.d. (mm) (c.v. %)	Range (g) (av. g)	s.d. (mm) (c.v. %)
W-5	4	123 - 159 (134.0)	16.6 (12.4)	115 - 156 (130.8)	18.3 (14.0)	61 - 130 (81.7)	32.7 (40.1)	51 - 118 (77.5)	29.0 (37.4)
B-5	4	63 - 144 (97.1)	34.7 (35.7)	66 - 129 (92.3)	26.4 (30.0)	8 - 97 (39.3)	40.4 (100)	9 - 71 (30.1)	27.6 (90)
W-50	25	126 - 163 (136.6)	17.5 (12.8)	110 - 159 (130.0)	21.0 (16.2)	66 - 141 (86.8)	36.0 (41.5)	41 - 131 (76.5)	38.4 (50.2)
B-50	25	69 - 128 (95.3)	24.5 (25.7)	58 - 124 (93.3)	28.4 (30.5)	11 - 67 (32.9)	25.4 (77.2)	6 - 77 (34.2)	33.2 (97.0)

[3] W: whole surface feeding; B: bottom surface feeding. "-5" and "-50" groups indicate low and high density conditions, respectively.
[4] Body sizes were estimated by using regression equation from one simultaneous measurement of the body length and the body breadth without anesthetizer.[7]
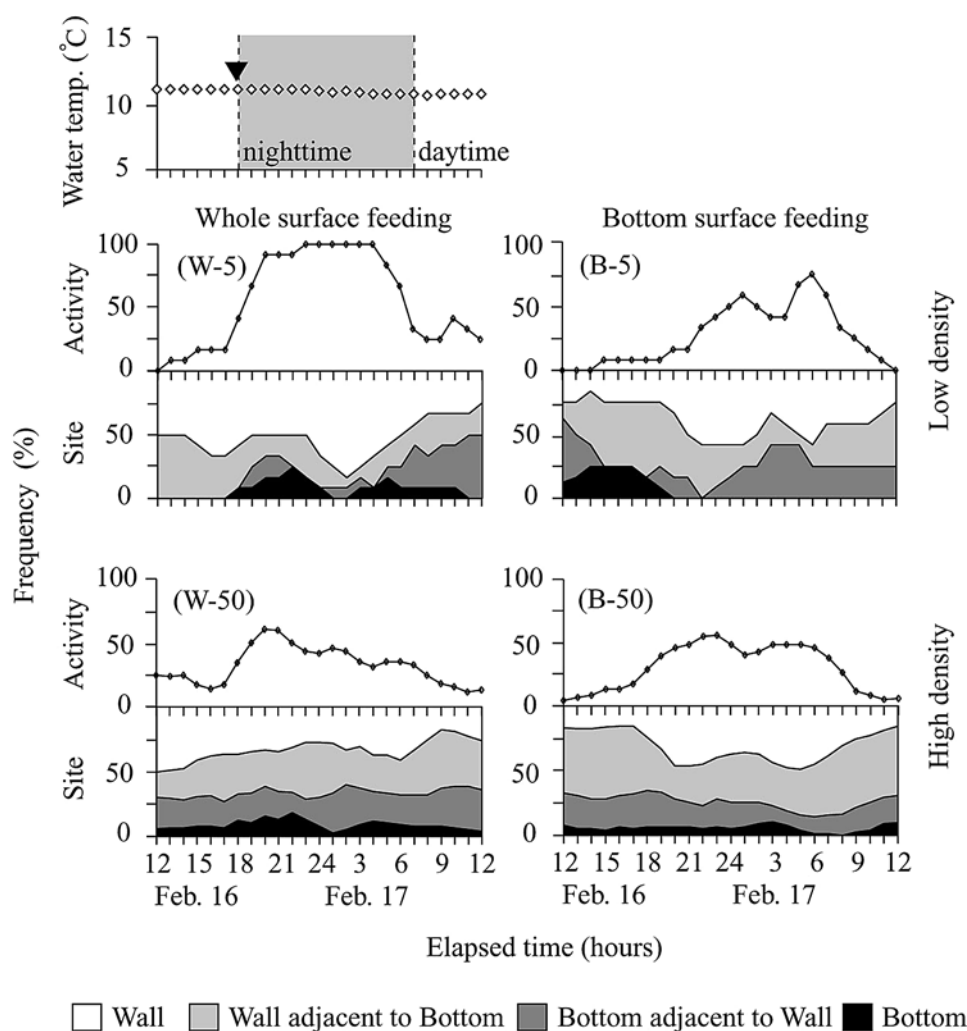[5] Body sizes were measured under the *l*-menthol anesthetizer.[8]

Fig. 5. Hourly percentages of daily activity (upper diagram) and attachment (lower diagram) of animals in experiment II (reduction of the uneven growth of *Apostichopus japonicus*) and hourly changes of water temperature in the rearing tank. Data are moving means over three size-classes intervals. Triangle indicates the time for feeding operation. Symbols indicate the same experimental groups as in Figure 4.

(variance : F-test, *Ps*＞0.05) (average : paired t-test, *Ps*＞0.05). The number of attachments at the border parts of walls and the bottom ranged 34-58% (av. 47.3%) and 65-78% (av. 73.5%) of the whole, in each of the wall and the bottom, respectively, and such position preference for these junctional areas was recognized.

In experiment II, we estimated of anesthetized body length as simple a measurement criterion of the body size while rearing period. In the present study, the estimates on the body length were not significantly different from the measurements (variance : F-test, *Ps*＞0.05) (average : t-test, *Ps*＞0.05), and thus the correction factor was not necessary for these estimates. Similarly, wet weights were not significantly different between the estimates and

measurements (variance : F-test, *Ps*＞0.05) (average : t-test, *Ps*＞0.05). In W-5, it could be confirmed that there were two animals had reproductive glands in early-maturing stage.

Discussion

In experiment I, there were some feces produced even in the non feeding condition, which was considered to result in the animals feeding activities upon the deposits of suspended material and diatoms. Six months after the start, survival rates of the animals were generally low, and comparison of the density effect could not be made. Therefore we compared the observation data at three months after the

start. The variance, namely, the degree of uneven growth was enhanced in the feeding condition regardless of the density, and was not in the non feeding condition. Under the feeding condition, the uneven growth in the high density (F-30-s, F-30-l) were caused by the high increments, which were attained by a few animals. The body sizes of these well-grown animals were close to that of the animals reared in low density and fed (F-5-s, F-5-l), and other animals showing minimal growth were close to the animals reared in high density and not fed (N-30-s, N-30-l). Therefore, the enhancement of the uneven growth in high-density conditions was considered to be a result of the inequality of the amounts of food, that explains the coexistence of a few satiated animals and other starving animals, probably caused by contingency on the frequency of meeting with food. The enhancement of the uneven growth was also observed in the low-density conditions (F-5-s, F-5-l), and it is suggested that the uneven growth might occur regardless of the density condition when there was inequality of the food amounts in the tank.

At the start of experiment II, the activity of the animals was low in the tanks with the whole surface feeding, where many bubbles attached to the mesh and the animals until about one week after the start. The reason of such bad condition of animals might be the physiological disorders caused by the contact with the bubbles. The cause of this trouble should be incompliance between the nylon mesh and seawater. If we soaked the mesh in seawater for about one week before use, this trouble could be avoided. And also, there was high mortality of 26-34% in the high-density conditions (W-50, B-50) within a month immediately after the start of rearing. Since this month was in the hot season and the mean temperature was as high as 27.3℃ in the present study, deterioration of water quality was suspected of the cause of high mortality. However, after this the mortality became lower and at last 50% of the initial animals survived in both conditions. In other reports, mortality of cultured animals in nursery conditions is generally high in the hot season, in the extreme case, mortality rates is as high as 98.1-99.6% within two months.[9] Thus, comparing the previous nursery culturing of *A. japonicus* and the present study, in which animals were reared for eight months, this 50% of mortality was not so high.

The whole surface feeding was superior in reducing the uneven growth compared with the bottom surface feeding (Fig. 4, Table 2). From this, unevenness of opportunity to eat on each attachment position, which led unevenness of food availability, was considered to be the largest cause for the uneven growth of *A. japonicus* in the rearing tank. Furthermore, the whole surface feeding was superior for the growth of juvenile *A. japonicus* compared with the bottom surface feeding. In the final (eight months after the start) measurements, average body size were ranked as follows: whole surface feeding-low density (W-5) > whole surface feeding- high density (W-50) = bottom surface feeding-low density (B-5) > bottom surface feeding-high density (B-50). However, for the average based on the four largest animals, this ranking was modified as follow: W-5 = W-50 > B-5 > B-50 (Table 3). Thus, W-50 and B-5 might appear the same ranking at the average of all animals, but W-50 was clearly superior in the production efficiency at the same space and food amounts. Also, since the average body size of well-grown animals of W-5 and W-50 were almost equal, there seemed to be surplus resources in W-5. Therefore, the growth of these well-grown animals in W-5 and W-50 might be the maximum growth under the rearing conditions of the present study, including water temperature, feeding frequency, food quality, and the tank capacity. Furthermore, the wet weights of these largest animals were close to the largest animal reported by Ito *et al.*[3] grown in a nursery pond of 5,000 m^2. Additionally, for final one month of the rearing period from February 26 to March 26, the growth rates generally declined in W-5, in which two animals formed the reproductive organs. Then, such decrease of the growth rate may be a general pattern of the growth in *A. japonicus* following the gonadal formation.

Throughout experiment II, the variances of body sizes among the tanks were not significantly different, in contrast to experiment I in which they were significantly different at three months after the start. For this reason, it was considered that none of the tanks of experiment II could keep uneven growth smaller than the tanks under the non feeding conditions of experiment I. Also, throughout experiment II, factors increasing uneven growth were observed in W-50 as large as in B-5, and it was especially

large increasing at eight month after the start（Table 2）. The factors increasing uneven growth was considered to be that constant food shortage occurred in the small animals, that were slow in feeding, in W-50 because of a sudden increase of feeding rates following the increase in size of well-grown animals as mentioned above. For example, during 25 hourly observations in February, the food that was attached to the walls in the evening was entirely dissipated by midnight, and the feeding frequency only once a day was clearly not sufficient. It seems that such an increase of uneven growth was derived from the feeding characteristics of *A. japonicus* under the rearing condition in the tank that was limited in capacity and food availability, and may not occur in nature.

For attachment positions, during the 25 hourly observations there were more animals attached to the walls than to the bottom of all tanks. However, when it was compared using the attachments per unit area, such a tendency was not seen. For example of such a selectivity of attachment position, it is widely noted about the abalone *Haliotis* （*Nordotis*）*discus discus* at rest during daytime.[10] *H. discus discus* prefer narrow spaces between near horizontal surfaces and attached upside down on the upper surface.[10] In the present study, active *A. japonicus* preferred the border part of the wall and the bottom regardless of time. Although it is quite probable that such a selection occurs, results of this experiment were not applicable to clarify this. It is necessary to carry out future studies with the detailed behavioral experiments.

It took nearly half an hour to do the daily work in the experiment II, in which one worker fed and cleaned once a day. On the other hand, it is not practical for large-scale nursery culture to use the whole surface feeding method using a brush like this study, because it will take a long time. In addition, such a feeding method was clearly insufficient in frequency of feeding. In the present study, the feeding activity of animals followed a diurnal pattern, and more than 40% animals were considered to be activity feeding at midnight. However, it is reported that the feeding actively of this species can occur nearly all day long, depending on changes in temperature and size of animals.[11,12] Therefore, in order to put this feeding method in practice, it is desirable to supply food automatically and continuous-

ly, and it is necessary to design more simple rearing method of a high efficiency including the feeding method and the clearing method. If it can be successful to feed nutritious food continuously under the suitable condition, it can be expected to obtain higher growth rate than that of the present study even in the large-scale nursery culture.

In the present study, the food used in both experiment I and II was considered to have no attractant effect for juvenile *A. japonicus*. This species has been thought to be a non-selective deposit feeder,[11] and its food attractant were still unknown. On the other hand, it was reported that adult *A. japonicus* accumulated to feed on chilled *U. pinnatifida*,[13] that the same food as the present study（experiment II）. In order to establish an ideal feeding method in practice, realization of a method that enables the animals to take in feed at their arbitrary positions is necessary（i.e., appropriate design of rearing tank and feeding method are problems that need to be solved）. However, it may be more realistic to research attractant substances for juvenile and investigate if the attractants can be added to the food or not. In future, we must research on this subject in parallel with feeding method research.

Acknowledgements

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水槽飼育における稚ナマコ*Apostichopus japonicus*の摂餌特性

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　飼育密度と給餌量を変えて中間育成試験をおこない，マナマコ種苗の成長差の成因を検討した。結果，飼育密度に関わらず，給餌をおこなうと一部の個体が急激に成長し，全体としての成長差が生じた。それ以外の個体の成長は無給餌状態の成長に近く，成長差の成因として，一部の個体の飽食と残りの個体の絶食が示唆された。次に，このような摂餌量の偏りが，摂餌機会の多少から生じると仮定し，給餌方法の改良を試みたところ，成長差は明らかに小さく抑えられた。以上より，種苗の成長差の成因は飼育水槽内の摂餌量の偏りであると考えられた。