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Diminution of Sea Cucumber *Stichopus japonicus* Juveniles Released on Artificial Reefs

Masataka Tanaka*

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In order to elucidate the cause of diminution of sea cucumber *Stichopus japonicus* juveniles released on artificial reefs, some experiments were performed at a breeding laboratory and a sea area with the six-months old juveniles classified into three classes as to size.

While the juveniles were picked up and carried to the release point, the handling hardly influenced their survival, regardless of body size. After the release of the juveniles into the sea, however, the small-sized juveniles (body length < 10 mm) inclined to die.

The juveniles were released on two artificial reefs that were covered or uncovered with a net fence in the sea, and the juveniles on the reefs decreased exponentially in number. The most principal cause of diminution of the juveniles released on both reefs was estimated to be mortality of the juveniles, especially small-sized individuals. The spontaneous migration of the juveniles during the long-term period, together with washout by tide, were related to the high degree of diminution of the juveniles on the uncovered reef.

Key words: sea cucumber, release, artificial reef, juvenile size, diminution, mortality, washout, migration

Sea cucumber *Stichopus japonicus* is one of the useful coastal fishery resources in various places including Ishikawa Prefecture.[1] As the catch of sea cucumber has recently tended to decrease, the release of artificially produced seeds have been carried out to increase the resources.[2] Many studies on the production techniques of sea cucumber juveniles were conducted,[3-18] which enabled us to produce a great deal of seed. However, little information has been reported on state of the juveniles after the release, such as growth, survival and behavior.[3-14, 19]

The follow-up survey of the juveniles after the release is difficult, because the appropriate tag or a mark is not found yet. Yanagisawa *et al.*[20] reported on the marking of sea cucumber juveniles by branding their epidermis with a hot Nichrome wire. According to the report, most of the marked juveniles could be distinguished three months later, but it seemed to be difficult to discern their marks four months later; this experiment was carried out in a laboratory but not in the sea. Fluorescent marking by dyeing the ossicle of the juveniles with tetracycline might be useful, which was distinguished half an year later.[11, 12] However, these marking are time-consuming, and it is also difficult to discern the mark after the catch.

The phenomena that a fair number of sea cucumber juveniles were not found in a short period after the release have been observed in some follow-up investigations.[3-12] We also observed the gradual diminution of the juveniles within a few days after the release on an artificial reef in the sea. Some reports indicated that the predation by sea star *Asterina pectinifera* led to the rapid disappearance of the juveniles.[3, 4, 6-10, 12, 21] On the other hand, Hamano *et al.*[19] suggested that the principal causes of the rapid disappearance were the oversight by the survey divers and the migration of the juveniles rather than the mortality by predators.

* 石川県水産総合センター技術開発部（〒927-0435 石川県鳳至郡能都町字宇出津新港 3-7）

In the course of our investigation, we observed that the epidermis of juveniles released on the reef (especially of small-sized individuals: < 7 mm body length) were dissolving.[10, 11] Those juveniles had little ability to adhere to substances because their tube feet were damaged. Therefore, a high mortality of the juveniles caused by dissolution of their epidermis and injury to their tube feet was assumed to be one of the causes of rapid diminution. Generally, when sea cucumber juveniles are transferred from nursery aquaria to the release point by truck, the juveniles are shaken. During the process, the juveniles might be damaged by handling. In order to ascertain whether a high mortality of the juveniles was produced by the handling before the release or the environmental factors after the release, a few experiments were performed at a breeding laboratory and in the sea, considering the body size of juveniles. Furthermore, to examine the main cause of the diminution, the follow-up investigation of the juveniles was carried out after the release on two artificial reefs that were covered or uncovered with a net fence.

Materials and Methods

Sea Cucumber Juveniles

Sea cucumber juveniles were raised in some aquaria at Ishikawa Prefectural Fisheries Research Center for about six months after artificial spawning from May 13 to June 2, 1997. The juveniles were picked up with a soft brush from the surface of polycarbonate corrugated plates (size: 30 × 40 cm, 1 set: 20 plates) used for attaching substance and from the wall and bottom of two different types of aquaria (1,000 *l* polycarbonate tank and 5,000 *l* FRP tank) from November 12 to 14. They were classified into three classes as to body size through two sieves (opening: 2 and 4 mm in diameter). One hundred juveniles were sampled from each class, and the body length was measured under anesthesia using 0.5 % menthol solution.[22] The others were held in 180 *l* aquaria under flowing sea water with aeration until experiments.

Experimental Methods

The juveniles were divided into 3 groups of 50 juvenile for each class, and they were put into each 1 *l* beaker with sea water fixed on the shaker (Yamato-kagaku Co. Ltd., IK41). The beakers were shaken in the following 3 periods: continual shaking of 60 min (60 min × 1); intermittent shaking of 40 min at intervals of 20 min (20 min × 2); intermittent shaking of 30 min at intervals of 10 min (10 min × 3). Thereafter each group was transferred into 180 *l* aquarium separately. After 15 days of raising period, the survivors were counted, and the mortality of each group was statistically compared with those of control group that were not under shaking.[23]

The juveniles that were held in 180 *l* aquaria for 3 days were picked up on November 18. Part of them were divided into 5 groups of 100 juvenile for each class and restored to the aquaria (Room group). They were picked up after 1, 3, 9, 16, and 24 days of raising, successively. The rest of them were held in 80 *l* polyethylene barrel containing sea water with aeration, and were carried to the seaside in Nanao North Bay at the middle of Noto Peninsula where it was about 40 kilometers from the fisheries research center for about 1 hour's driving (Fig.1). Part of them were divided into 5 groups of 100 juvenile for each class, and were put in baskets containing fist-sized stones that were provided from a quarry (Sea group). Each basket was enclosed with 18-

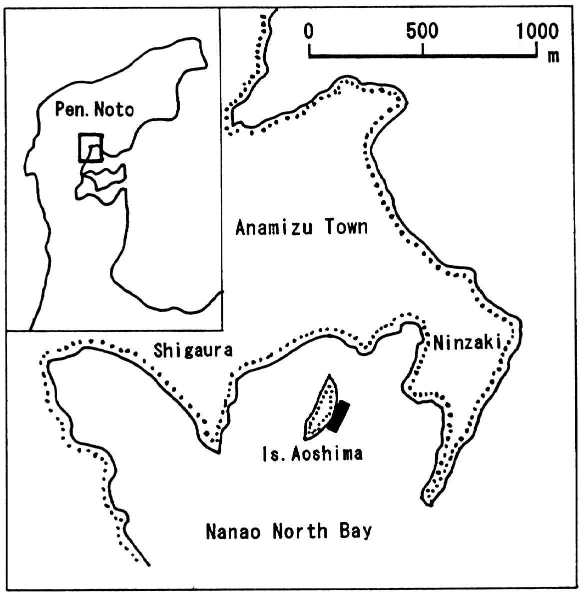


Fig. 1. Map showing the locality of Aoshima Island in Nanao North Bay. Solid line indicates the experimental site.

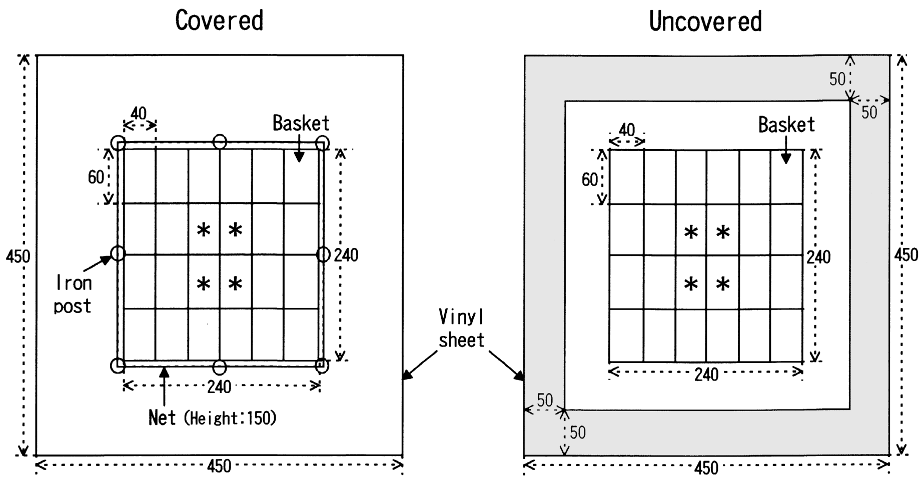


Fig. 2. Size of covered and uncovered artificial reefs used for the follow-up survey of released sea cucumber juveniles. Asterisk in both reefs shows the release points. Figure is given in centimeter.

mesh polyethylene net (*ca.* 1.2 mm mesh) and placed into the sea, and the survival number was checked 1, 3, 9, 16, and 24 days after. Another part of them were carried back to the breeding laboratory and divided into 5 groups of 100 juvenile for each class (Carry group). They were held in the aquaria again and picked up after the same raising periods as Room group. The data of the survival on each group were statistically compared regarding each class.[23)]

Two artificial reefs were built to conduct a follow-up survey on the survival of sea cucumber juveniles in the sea in Anamizu Bay on October 23, 1997 (Fig.1). Twenty four baskets (60 × 40 × 20 cm) containing above-mentioned stones were arranged in 4 files and 6 ranks upon vinyl plastic seats (450 × 450 cm) at a depth of 1 meter or less (Fig.2). One of them was enclosed with a net fence (150 cm high, *ca.* 1 mm mesh) bound to 8 iron props with rope (Covered reef). The other was opened, and a number of stones were piled up 50 cm wide outside the reef (Uncovered reef). The juveniles carried to the seaside as mentioned above on November 18 were divided into 2 groups of 2,400 juvenile consisting of 800 juvenile each for 3 size classes (large, middle, and small). They were transferred to the reefs and released softly upon 4 baskets in the middle of each reef with a net. Half of the baskets in each reef were lifted up alternately on a working raft 1, 3, 9, 16, 24, and 50 days later. The juveniles stuck on stones in each basket and on the bottom of reefs after removing of the baskets were gathered into buckets separately. Surface of the vinyl plastic seats was checked by diving with a mask and a snorkel so as not to overlook the juveniles. They were transferred to a workplace by the seaside and anesthetized with 0.5 % menthol solution to measure their body length for each basket. The baskets were returned to the original positions, and the juveniles were released again on the same baskets as they had existed. The outside stones of the uncovered reef were also checked whether the juveniles moved out from the reef. Since the observation after 9 days, the juveniles stuck on the outside stones were also picked up and their body length were measured in the same manner as mentioned above. Based on the results of the follow-up survey, the survival process and the cause of the diminution of juveniles were estimated.

Results

Body Length of Sea Cucumber Juveniles

The compositions of body length of sea cucumber juveniles used in this study were shown in Fig.3. The average body length of large, middle, and small classes, was 32.9 mm, 14.4 mm, and 6.9 mm, respectively. The large, middle, and small classes consisted of individuals with the body length of 10 mm and upward, 5 to 25 mm, and 15 mm or less, respectively. The mode was 25 to 30

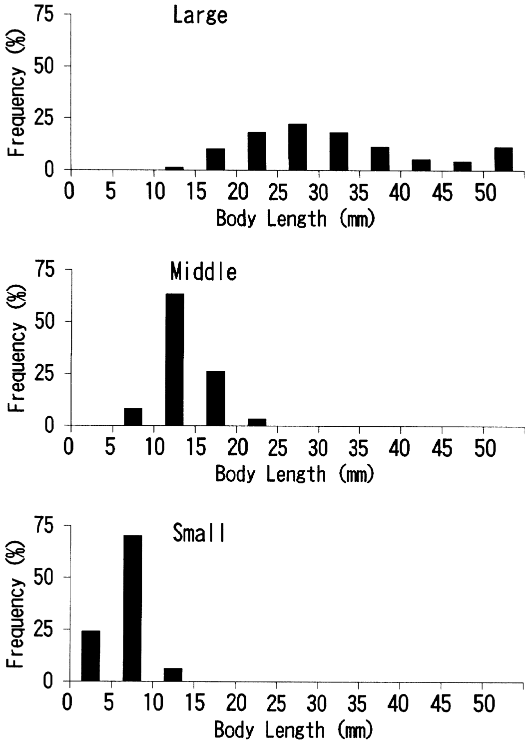


Fig. 3. Body length composition of three groups of sea cucumber juveniles.

mm in large class, 10 to 15 mm in middle class, and 5 to 10 mm in small class.

Influence of Shaking and Handling on Mortality

The survival numbers of three classes under each shaking conditions were compared with those of control that were not under shaking (Fig.4). The survival number decreased in small group under 60 min × 1 shaking, and the significant difference against control was indicated at 1 % level. The other groups showed high survival rates, and the significant differences against control were not indicated.

Figure 5 shows the changes of survival number classified into the body size and handling way for 16 days from the beginning of the experiment. The results of the statistic analysis were also shown in Table 1.

In large class, all the juveniles survived regardless of the handling ways. In middle class, though the significant differences were indicated between sea group and carry group, and between sea group and room group on day 1, the survival rate was generally high. In small

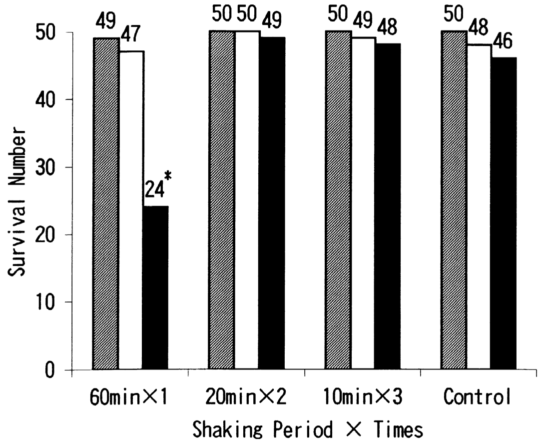


Fig. 4. Survival number of sea cucumber juveniles which received each shaking operation. The number was counted after 15 days. □: large, ▩: middle, ■: small.
* Significant difference between survival in small group of 60 min × 1 and those of control is shown ($p < 0.01$).

class, the number of survival on sea group was less than those on carry group or room group during the experimental period of 16 days.

In sea group, the number of survival on small class was less than those on large class and middle class for 16 days. There were significant differences indicated between small class and large class, and between small class and middle class on all observation days. In carry group, the number of survival on small class was less than those on large class and middle class on day 3, 9, and 16, and the significant differences were indicated between small class and large class at the same days. In room group, the number of survival on small class were significantly less than those on large class and middle class on day 16.

Follow-up Survey of Juveniles Released on Artificial Reefs

Figure 6 shows the number and average body length of the juveniles found in each position of the baskets and outside stones of two artificial reefs. Though most of the juveniles were found in the center of the reefs on day 1, they were found in all positions within a few days. Thereafter, more juveniles became observed in the direction of the island with the elapse of day in the covered reef. Total numbers of the juveniles found in the

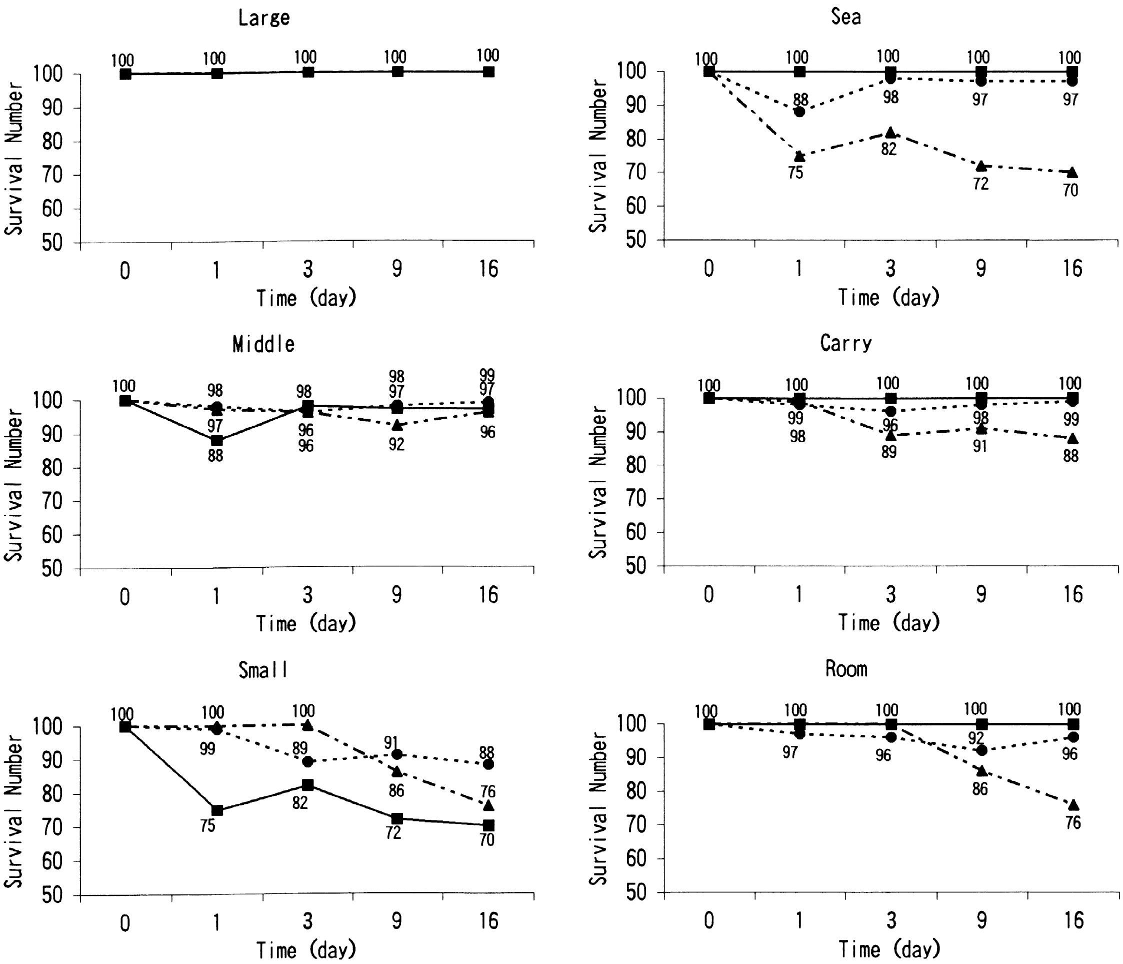


Fig. 5. Changes in survival number of sea cucumber juveniles with different classes of body size (left figure), and with different ways of handling (right figure). ■: sea, ●: carry, ▲: room (left figure), ■: large, ●: middle, ▲: small (right figure).

covered reef were more than those in the uncovered reef on all observation days, and the numbers decreased as the day progressed in both reefs. The average body length of the juveniles found in the covered reef was high compared with that in the uncovered reef on all observation days. The average body length of the juveniles on the outside stones was higher than that in the inside baskets in the uncovered reef since the observation after 9 days.

Figure 7 shows the changes in the body length composition of the juveniles during the survey. The ratio of the individuals with the body length of 5 mm or less and that of 10 mm or less on the release day was 8.0 % and 34.0 %, respectively. The ratio of the individuals with the body length of 10 mm or less, which almost consisted of small class (Fig.3), showed a tendency to decrease as the day progressed in both reefs. On the other hand, the ratio of the individuals with the body length of 15 mm or more, which consisted of middle and large classes (Fig.3), had a increasing tendency as the day progressed in both reefs.

The estimated survival number on each observation day on both reefs were plotted on Fig.8. Data in this figure were shown as the value which doubled actual counted numbers on day 1, 3, 9, and 14 in the uncovered reef and on all observation days in the covered reef, and as the value which multiplied actual counted numbers by 4 on day 24 and 50 in the uncovered reef, because the buckets in which juveniles were put from the uncovered reef were swept away by a high wave in the midst of the observation on day 14. The diminution processes of the released juveniles on both reefs were approximated by exponential function with high correlations.

Table 2 shows the estimated survival and diminished which were revised by the exponential function curve on two reefs. During 50 days after the release, the survival number was estimated to decrease to *ca.* 1/4 in the covered reef and to *ca.* 1/10 in the uncovered reef.

Table 1. Significant differences test between two data of the survival on each elapsed day in each class of body size of the juvenile (A) and in each group of handling of the juvenile (B)

		Time Elapsed (day)			
		1	3	9	16
Class	Relation (A)				
Large	S-C	N.S.	N.S.	N.S.	N.S.
	S-R	N.S.	N.S.	N.S.	N.S.
	C-R	N.S.	N.S.	N.S.	N.S.
Middle	S-C	*	N.S.	N.S.	N.S.
	S-R	*	N.S.	N.S.	N.S.
	C-R	N.S.	N.S.	N.S.	N.S.
Small	S-C	**	N.S.	**	**
	S-R	**	**	*	N.S.
	C-R	N.S.	**	N.S.	*
Group	Relation (B)				
Sea	L-M	**	N.S.	N.S.	N.S.
	L-Sm	**	**	**	**
	M-Sm	*	**	**	**
Carry	L-M	N.S.	N.S.	N.S.	N.S.
	L-Sm	N.S.	**	**	**
	M-Sm	N.S.	N.S.	N.S.	**
Room	L-M	N.S.	N.S.	*	N.S.
	L-Sm	N.S.	N.S.	**	**
	M-Sm	N.S.	N.S.	N.S.	**

S: sea, C: carry, R: room, L: large, M: middle, Sm: small.
* Significant at $p < 0.05$.
** Significant at $p < 0.01$.
N.S.: not significant ($p > 0.05$).

Discussion

The phenomena that a fair number of sea cucumber juveniles were not found in a short time after the release have been observed in some follow-up investigations.[3-12] We observed some juveniles, especially small ones, whose epidermis was dissolving and tube feet were injured after release in the course of our previous survey.[10-11] Therefore, we supposed that a high mortality of small-sized juveniles resulting from a decline in their vitality mainly caused the diminution. Moreover, it seemed that the juveniles were healthy during the breeding period and were damaged after the release. Thus we considered that the handling of the juveniles before the release was related to high mortality.

We examined the extent of mortality resulting from the damage of their tube feet by shaking the beaker keeping juveniles at the laboratory, assuming that the vibration in transit to a releasing point led to lowering of their ability to adhere. The survival numbers of small class under 60 min × 1 shaking were significantly less than those of control. However, a number of *Harpacticoida*, order of copepod, bred in the aquarium in the latter of the examination. This copepod is informed to be harmful to sea cucumber, because the copepod attaches to the epidermis of sea cucumber and bites the surface.[24] Therefore, the above-mentioned decrease is possibly due to the breeding of the copepod but not to the damage of their tube feet under vibration. The vibration in transit presumably did not influence the subsequent survival of the juveniles.

As a result, the decline of vitality of juveniles after release was probably caused by the environmental influence of the sea irrespective of handling before release. Using the juveniles which were picked up from rearing aquaria and transported by a truck, we compared the survival of the juveniles in the sea with that in the aquaria in consideration of body size. The large- and middle-sized juveniles survived in the sea during the experimental period of 16 days, while the survival number of the small-sized juveniles decreased to 70 % after 16 days. The mortality of the juveniles which were restored to the aquaria after the pickup or were carried back from release point to the breeding laboratory was lower than that of the juveniles put in the sea. Accordingly, it seemed that the pickup from breeding aquaria and the truck conveyance with vibration did not influence survival of the juveniles after the release, regardless of body size. For small-sized juveniles, however, some factors to drive them to death would be in the natural environment after the release.

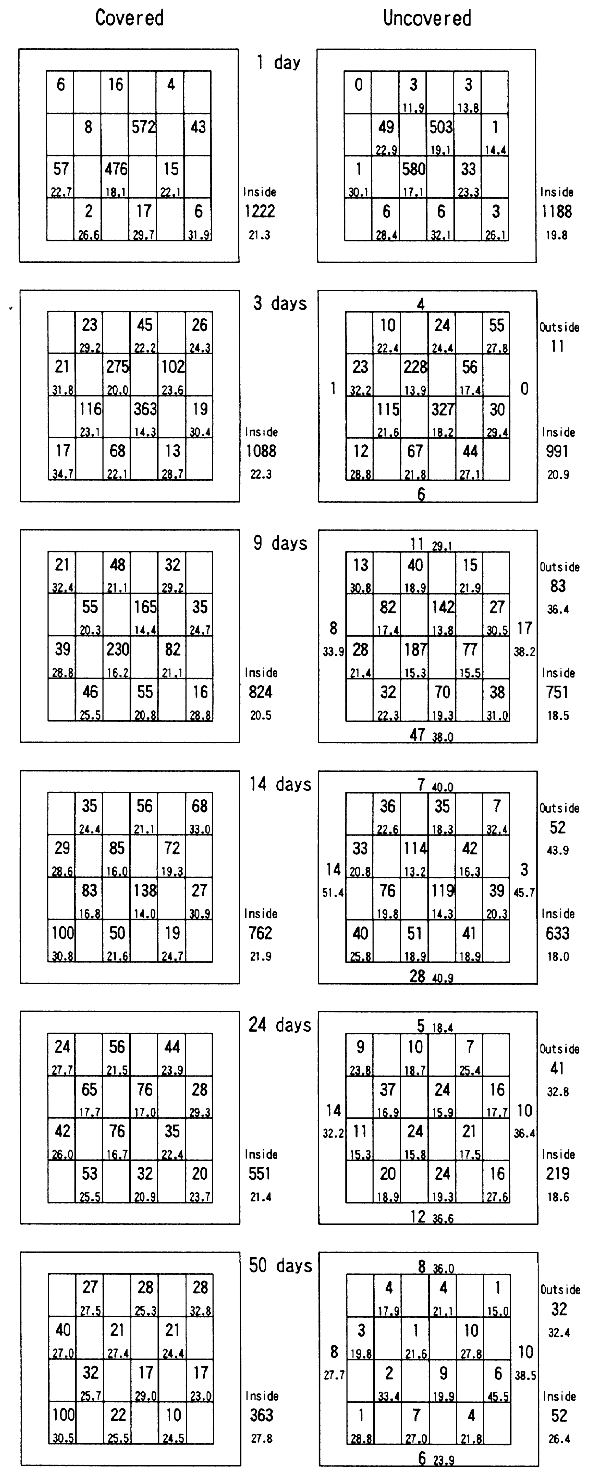


Fig. 6. Distribution of sea cucumber juveniles after release on the covered and uncovered reefs. Large figure indicates the number of juveniles found in each basket or outside. Small figure indicates the average body length (mm) in each basket or outside. Large and small figures outside the frames indicate total number and total average body length (mm), respectively.

Through the practical follow-up survey after release in the artificial reefs, the diminution of the juveniles found after the release was shown to be mainly due to the death of juveniles. Approximately 100 % of the juveniles were found in the covered and uncovered reefs in the 1 day after the release. Therefore, oversight of the juveniles by survey divers[19] was negligible under the present experimental conditions. Nevertheless, the number of the juveniles found after release decreased rapidly in the covered reef. The diminution was presumed to be due to a high mortality of the small-sized juveniles on the basis of the results that the small-sized juveniles tended to die in the sea. According to the histogram on the body length of juveniles found out in both reefs, the rate of the juveniles with the body length of less than 10 mm decreased gradually after the release in both reefs. It is reasonable to consider that the disappearance of small-sized juveniles was the major cause of decrease of total numbers in the reefs. The difference between the number of juveniles found in the covered reef and that in the uncovered reef indicates the existence of the juveniles which were washed out by tide or migrated outside spontaneously in the uncovered reef. In the present study, (1) average body length of the juveniles in the uncovered reef was higher in the outside stones than in the inside of the reef, (2) average body length of juveniles was higher in the covered reef than in the uncovered reef, and (3) the average body length was higher in the exterior baskets than in the central baskets. Those results suggest that larger juveniles tended to migrate outside the reefs. On the other hand, the juveniles washed out with tide were thought to be mostly composed of small-sized individuals, because the small-sized individuals had a low ability to adhere on substances.

Hatanaka *et al.*[21] suggested that predation of the juveniles by sea star *Asterina pectinifera* was one of the causes of rapid disappearance. Some sea stars captured inside of the uncovered reef were fixed in 10 % formalin and carried to our laboratory on this follow-up survey. However, sea cucumber and their ossicle were not found in their stomachs (data not shown). This finding indicates that the predation by sea star might be one of the causes of diminution during long-term period, but

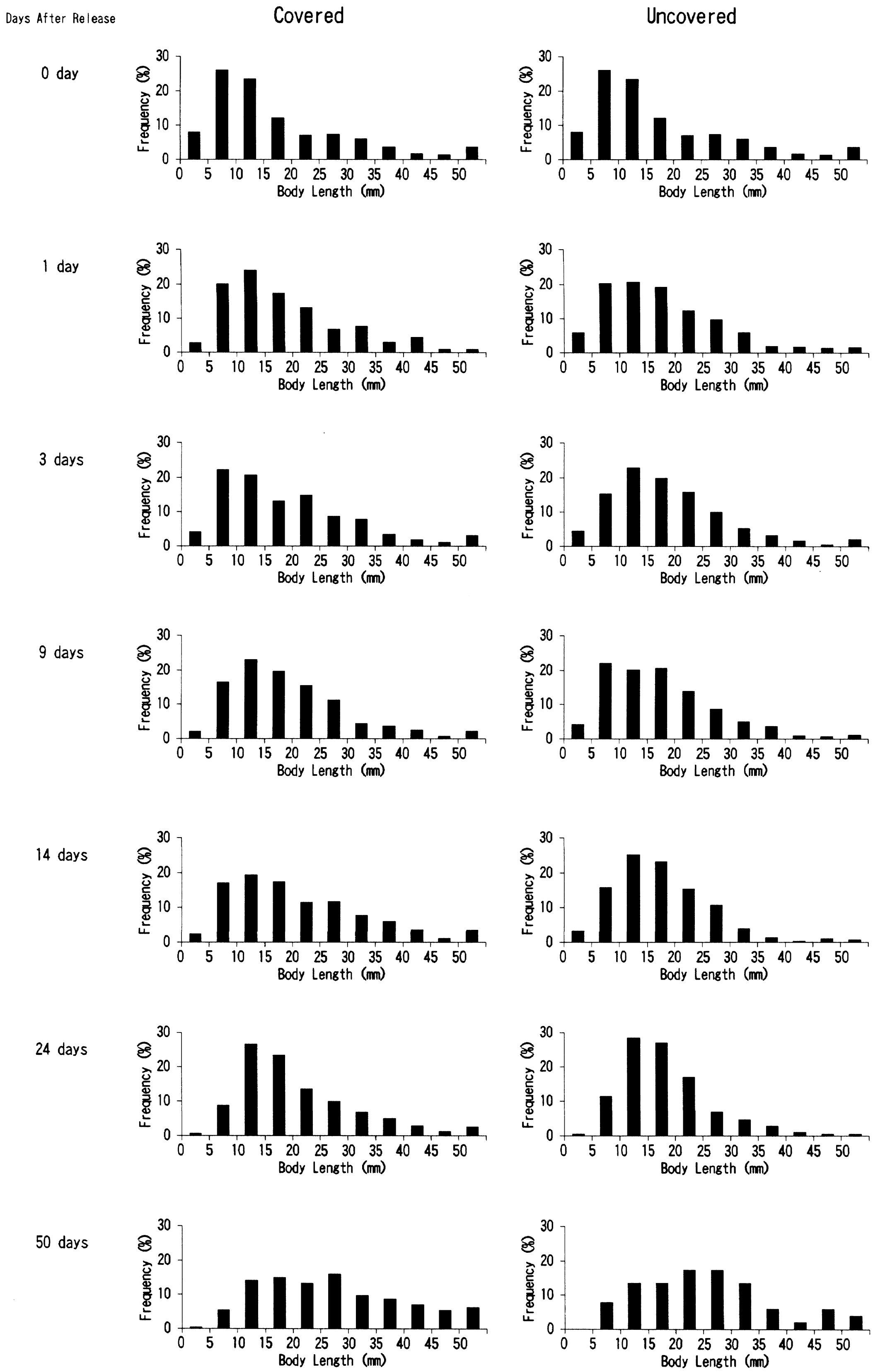
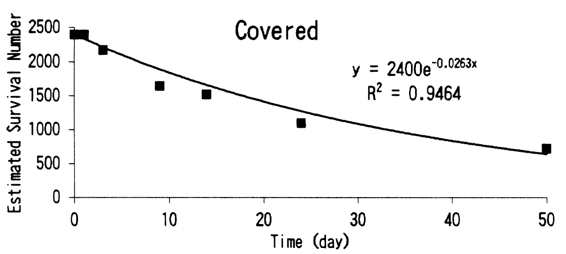


Fig. 7. Histogram of body length after the release on covered and uncovered reefs.

Table 2. Changes in estimated survival and diminished numbers in the covered and uncovered reefs

Time (day)	Covered Reef			Uncovered Reef				
	Survival	Diminished		Survival	Diminished			
		Total	Mortality		Total	Mortality	Migration	Washout
0	2400	0	0	2400	0	0	0	0
1	2338	62	62	2288	112	62	0	49
3	2218	182	182	2081	319	180	11	129
9	1894	506	506	1564	836	483	94	259
14	1661	739	739	1233	1167	676	146	346
24	1277	1123	1123	766	1634	961	187	486
50	644	1756	1756	222	2178	1340	219	619

The numbers are revised by the exponential function curve in Fig.8. The number of mortality in the uncovered reef is aggregate of the calculated value by the following expression: A/B×C (A: the number of mortality obtained from the previous observation in the covered reef, B: the number of estimated survival of the previous observation in the covered reef, C: the number of estimated survival of the previous observation in the uncovered reef). The number of spontaneous migration in the uncovered reef is aggregate of the actual counted number on the outside stones of the reef. The rest of diminished number except mortality and migration in the uncovered reef is conjectured to be the number of washout.



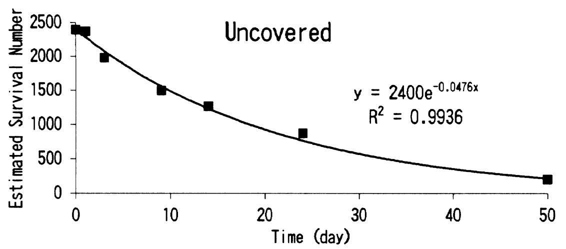


Fig. 8. Changes in estimated number on the covered and uncovered reefs. Curved lines on both reefs show exponential approximate curves.

not a major cause of diminution.

According to the results of this survey, the high mortality of small juveniles (less than 10 mm in body length) which had a low vitality in the sea and the washout of the small juveniles by tide were highly associated with the diminution of the sea cucumber juveniles in the early period after the release. In order to increase the survival rate, it is necessary to release large juveniles whose body length is more than 15 mm because comparatively large juveniles hardly die in the early period after the release. At present, however, it is technically difficult to produce a number of large juveniles in a short-term, because about half a year is needed for the juveniles to reach the average body length of more than 15 mm, and the variety in size of juveniles is remarkable.[12)] Incidentally, in this study, the juveniles were repeatedly picked up from the reef, anesthetized, and returned to the reef after measurements. Moreover, though the place where the juveniles were released was seemed to be appropriate as Hamano *et al.*[25, 26)] pointed out that the tidal zone was suitable area for the juveniles, the net of the covered reef was gradually changed to be muddy. Namely, the conditions of the juveniles and the reefs in the latter period were considered to be quite different from those in the initial stage. The effects of some artificial factors on the results of this survey were presumably unavoidable, nonetheless the mortality of the juveniles was notable. We need to investigate internal factors such as physiological properties besides external factors in the sea to elucidate the causes of mortality of the juveniles after release.

Acknowledgments

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