Vertical distribution of juvenile sea cucumber *Apostichopus japonicus* in a mesocosm experiment

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ABSTRACT
Early life history of the sea cucumber *Apostichopus japonicus* is important for developing methods for stock management and enhancement. However, information on the ecology of juvenile sea cucumbers in the wild is scarce, partly because of their low density. We investigated their diel change in vertical distribution in an adventitious mesocosm containing juvenile sea cucumbers in the yard of the Maizuru Fisheries Research Station, Kyoto University. The density of *A. japonicus* juveniles in shallow water was higher than in deep water. The profiles of the water temperature and salinity were relatively uniform throughout the water column, while photon strength was higher in shallow water in daytime. This result shows that juvenile *A. japonicus* sea cucumbers have negative geotaxis despite having negative phototaxis. In field observations in Maizuru Bay, there were no juveniles at shallow sites, such as shores, suggesting that other environmental factors prevent juveniles from moving to shallower areas.

KEYWORDS: echinoderm, geotaxis, phototaxis

INTRODUCTION
Spatial distribution of sea cucumbers is known to be depth-dependent. In some species of sea cucumbers, the larger the individuals, the more abundant they are in deeper zones (Chou 1963; Conand and Byrne 1993; Hamel and Mercier 1996). On the other hand, the size–depth relationship of other species is not clear (Mercier et al. 2000). Moreover, information regarding the depth-dependent distribution of sea cucumbers at the juvenile stage is scarce (Long et al. 1996; Mercier et al. 2000; Slater and Jeffs 2010) not only in the wild but also in captive rearing conditions. The Japanese common sea cucumber *Apostichopus japonicus* is the most commercially valuable species among sea cucumbers (Akamine 2005; Choo 2008). Various techniques for farming them have been improved in recent years (Yang et al. 2015), but information on their ecology in the wild is rare, particularly at the juvenile stage. In this study, we investigated the vertical distribution of juvenile *A. japonicus* at an adventitious mesocosm in the yard of the Maizuru Fisheries Research Station (MFRS), Kyoto University, Japan.

MATERIALS AND METHODS
We conducted the study at a settling tank in the yard of MFRS, located in Maizuru city, Kyoto, Japan (Fig. 1, 35° 29.4 N, 135° 22.1 E). This tank was used for the settlement of sediment particles in natural seawater before filtration. Seawater in this tank overflows through the drainpipe to the next filtration tank. Seawater was taken from the adjacent sea area of MFRS in Maizuru Bay, and the mouth of the uptake hose was set at 5 m depth from the float of the observation pier. We used this tank as a mesocosm to examine the yearling individuals of *A. japonicus.* There were many sea cucumbers recruited to this tank during their planktonic period through the uptake hose. We considered them to be yearlings because this tank was washed (drying and scrubbing attached organisms) in May 2014, when it was confirmed to be the spawning season of *A. japonicus* around this region.

Vertical position and abundance of juvenile sea cucumbers on the sidewall of the mesocosm were recorded using a water-resistant camera. This camera was attached to the end of the polyvinyl chloride pipe (length: 150 cm) and fixed 50 cm from the wall to keep the subject area constant (Fig. 2a). We recorded photos of the sidewall and did not record that of the bottom, as the bottom surface was filled with soft sediment mainly consisting of settled particles and juvenile sea cucumbers were difficult to distinguish in the photos. The trials were performed from 6 positions on the sidewall (A to F, Fig. 1c) at 6 depths (0.2, 0.4, 0.6, 0.8, 1, and 1.2 m) 4 times per day (06:00, 12:00, 18:00, and 00:00) over the course of 3 days (Dec 21 2014, Dec 29 2014, and Jan 11 2015) using one set of the camera apparatus. Water temperature and salinity were measured by a conductivity temperature depth profiler AAQ 1183 (JFE Advantech Co. Ltd, Nishinomiya, Japan), and these profiles were confirmed to be relatively uniform (data not shown). Photon flux density was measured using a photon flux detector of DEFI-L (JFE Advantech Co. Ltd) on Dec 20 2014 and Jan 11 2015 concurrently with taking photos.

Abundance of the sea cucumber juveniles was determined from the photos, but only large enough individuals that could be distinguished as a sea cucumber were counted. More than 95% of the sea cucumbers observed while diving in Maizuru Bay were *A. japonicus* including both the green and red morphs (R. Masuda, unpubl data). Some individuals were confirmed as *A. japonicus* by direct observations at the mesocosm during the experiments. Therefore, we regarded all juveniles of sea cucumbers in this tank as *A. japonicus*.

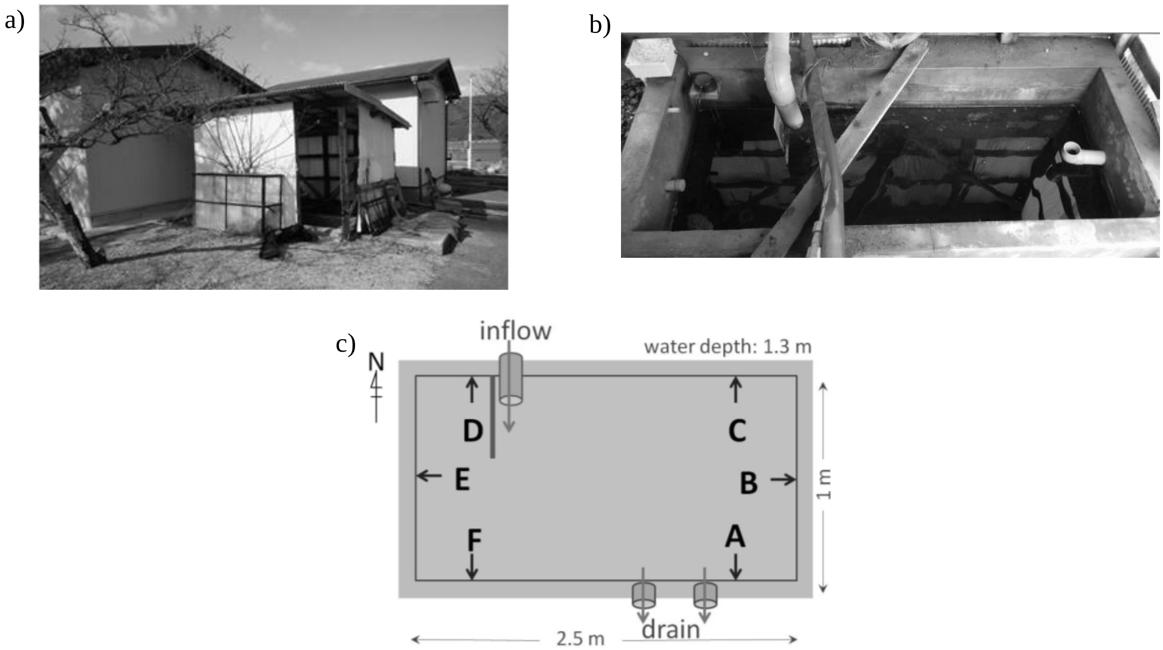

Fig. 1 Settling tank as a mesocosm at Maizuru Fisheries Research Station (MFRS), Kyoto University. a) Outlook of the tank shed. b) Aspect of the tank. c) Schematic drawing of the tank. A–F indicate the positions of the waterproof cameras recording from the wall. Each arrow with A–F indicates the camera angle.

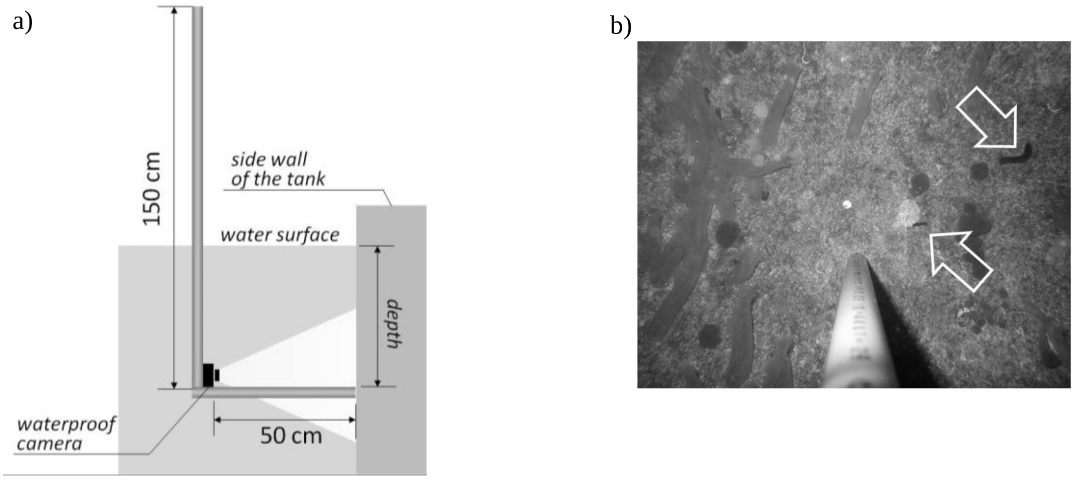

Fig. 2 Snapshots of juvenile sea cucumbers, *Apostichopus japonicus*. a) Schematic drawing of experimental setup. b) An example of snapshots. Two individuals (indicated with arrows) were confirmed from this picture.

Statistical analysis was conducted using generalized linear models (GLM, with a Poisson error structure and log link function). Indicators of the number of juveniles per photo were evaluated by the explanatory variables of time, depth, and position using model selection based on AIC (Akaike Information

Criterion) after considering the variance inflation factor. All statistical analyses were performed in R 3.0.2 (R Core Team 2014) with 'MASS' and 'gplot' packages.

RESULTS AND DISCUSSION

In the mesocosm, water temperature and salinity ranged from 12.3 to 14.6 °C and 30.7 to 33.2, respectively, throughout the experiment. Photon flux density was measured at two trials, and the data for 12:00 are shown in Table 1. For the other times (06:00, 18:00 and 00:00), the values of the photometer were under the detection limit of 1.5 μmol/ (m^2 s). Abundance of *A. japonicus* juveniles on the sidewall of the mesocosm ranged from 0 to 7 individuals per photo, with a 0.1875 m^2 subject area (Fig. 3). Estimated anesthetized body length of *Le* (Yamana and Hamano 2006) of the observed juveniles ranged between 9.80 and 91.83 mm, and the average *Le* was 35.29 mm.

Table 1 Photon flux density (μmol/(m^2 s)) on the side wall of the settling tank at each depth and position at 12:00 on a) Dec 29 2014 and b) Jan 11 2015.

a)

depth (m)	*position*					
	A	B	C	D	E	F
0.2	7.95	5.30	26.37	5.73	11.20	8.20
0.4	6.70	5.13	18.97	6.80	8.37	8.30
0.6	5.60	4.73	12.77	8.13	7.97	8.23
0.8	4.80	4.07	10.20	7.77	7.23	7.60
1	4.40	3.13	7.17	7.53	6.90	7.05
1.2	4.10	2.50	5.83	8.13	6.33	5.57

b)

depth (m)	*position*					
	A	B	C	D	E	F
0.2	8.95	14.20	29.45	7.70	9.78	10.83
0.4	7.95	13.13	21.23	7.53	8.48	8.23
0.6	7.43	7.48	15.85	7.30	5.80	6.90
0.8	7.20	5.88	13.03	7.33	4.58	5.63
1	6.00	4.50	8.53	7.53	5.20	4.83
1.2	5.13	3.38	6.98	6.48	4.55	4.53

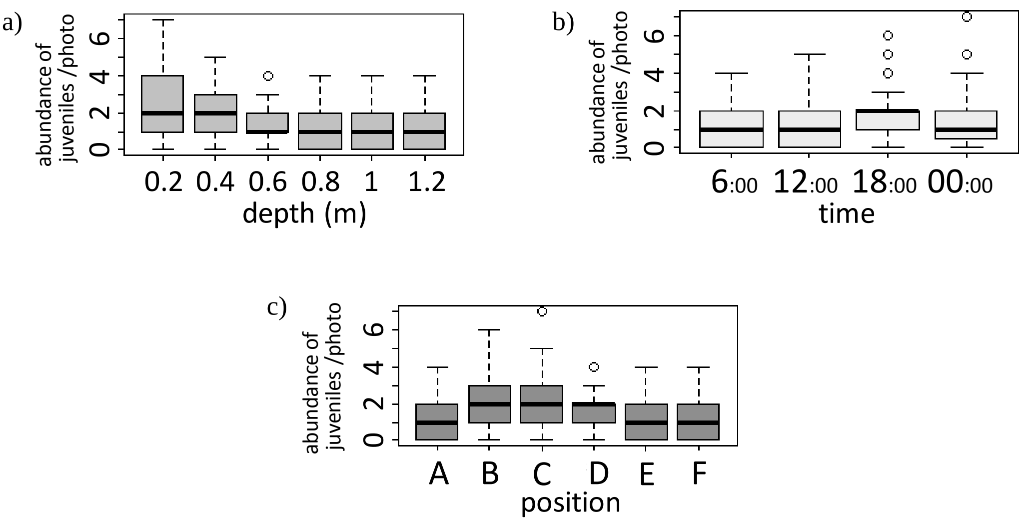

Fig. 3 Abundance of *Apostichopus japonicus* juveniles with the three variables: a) water depth b) time and c) position in the settling tank. Open circles indicate outliers which are defined as the data over the third quartiles + 1.5 interquartile range of each dataset.

Results of the GLM based on AIC showed that three variables showed a correlation with the abundance of juveniles (Table 2). Water depth had a negative correlation, while time had a negative correlation at 12:00 and positive correlation at 18:00 and 00:00. Position had a positive correlation at positions B, C, and D and negative at E and F compared to position A. In the constructed model, over dispersion was not confirmed (dispersion parameter was 1.07 (Zuur et al. 2009)). The results of the statistical analyses are discussed in detail below.

Table 2. Summary of Generalized Linear Models for the abundance of juvenile *Apostichopus japonicus* in a mesocosm based on AIC. a) ΔAIC of all constructed models. b) Estimated values of the best fitted model.

a)

Variables in models	*df*	ΔAIC
depth + time + position	10	0
depth + position	7	0.24
depth + time	5	16.50
depth	2	16.74
position + time	9	70.85
position	6	71.09
time	4	87.36
null	1	87.59

b)

Variables	Estimate	Std. Error	z value
(Intercept)	0.869	0.141	6.144
depth	-0.986	0.118	-8.386
time			
12:00	-0.079	0.115	-0.688
18:00	0.179	0.108	1.662
00:00	0.091	0.110	0.824
position			
B	0.348	0.133	2.613
C	0.377	0.133	2.847
D	0.223	0.137	1.630
E	-0.134	0.149	-0.894
F	-0.065	0.147	-0.440

1) Water depth effect: The results of statistical analysis show that juvenile *A. japonicus* were more abundant in shallower zones than in deeper zones (Fig. 3a). This means that juveniles showed negative geotaxis in the mesocosm. Moreover, Table 2a shows that all of the top-ranked models contain depth variables, indicating that the water depth is the most important factor in the distribution of *A. japonicus* juveniles in this mesocosm.

There are few reports on the distribution of juvenile sea cucumbers in the field. One of such reports about the vertical distribution of the juvenile sea cucumber *Cucumaria frondosa* showed that larger individuals prefer to inhabit deeper waters (Hamel and Mercier 1996). However, to our knowledge, there are no reports of the shallow residence of juveniles in the field, such as near shores. These facts and the vertical distribution in this mesocosm suggest that other environmental factors, such as waves, strong light, and/or exsiccation, suppress the behavior of the juveniles to crawl to the shallower sites in the field. The mechanisms of vertical distribution of juveniles in the present study were not identified, but there are several candidates that may control such depth-dependence (*e.g.*, negative phototaxis (Yamana et al. 2009; Dong et al. 2010; Yamaguchi et al. 2016), food resources (Slater and Jeffs 2010), and/or stable environment (Mercier et al. 2000)).

2) Time effect: Adult and juvenile *A. japonicus* are known to have negative phototaxis (Yamana et al. 2009; Dong et al. 2010; Yamaguchi et al. 2016). In the daytime, photon flux density was higher in the upper area of the side wall (Table 1), and juveniles in this mesocosm tended to move down to deeper zones including the bottom area to avoid daylight at 12:00, as compared to that at the other times (Fig. 4a). Therefore, the density of juveniles on the sidewalls probably decreased, while we did not count the number of juveniles at the bottom.

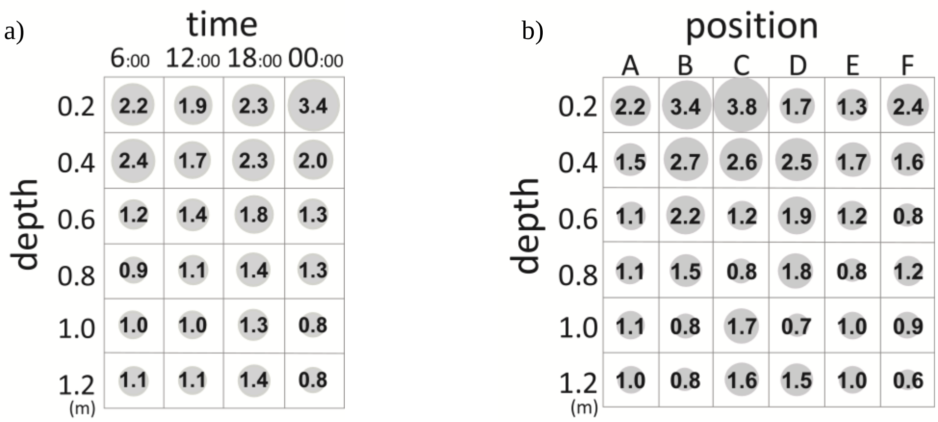

Fig. 4 Multivariate plots of *Apostichopus japonicus* juvenile abundances using balloon plots. a) Time-depth b) position–depth correlation with juvenile abundances. The bubble sizes and the number in each bubble represent the mean density of juveniles per photo.

3) Positions: Figures 3c and 4b show that juveniles were abundant at positions B, C, and D. Table 1 shows that the photon flux density at the shallower zone of position C was higher than that of the other positions on both days, and that of position B was higher than those of A, D, E, and F on Jan 11 2015. Juveniles showed

negative phototaxis as discussed above, while the total density at positions B and C was higher than that at A, E, and F. These facts indicate that the amount of available feeds on the surface of the sidewall may be rich at positions B and C, resulting in higher juvenile density at these sites.

On the other hand, high values of photon flux density were not confirmed at position D. Another reason for the high density of juveniles at positions B, C, and D may be explained by the flow velocity at each position. The water current at positions B, C, and D seems to stagnate because these positions were out of the axis of inflow to drainage. However, the actual velocity was not measured in this study.

In this study, we showed that juvenile *A. japonicus* have negative geotaxis within a mesocosm, although no juveniles were observed at the shallow sites in Maizuru Bay during our field surveys. To elucidate the suitable environments as nursery grounds for sea cucumber juveniles, effects of environmental factors, such as depth, vegetation, light intensity, food availability, and protection from predators, on juvenile distribution are important topics for future research.

ACKNOWLEDGMENTS

We thank the staff of the MFRS and two anonymous reviewers for their useful comments. This study was partly supported by the Coastal Ecosystem Complex Project of the Ocean Resource Use Promotion Technology Development Program, MEXT of Japan.

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