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# Spatio-temporal variability in the recruitment of a Western Pacific sand dollar population

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## Abstract

A sand dollar *Scaphechinus mirabilis* inhabits the intertidal and subtidal sandy bottom of temperate Northwest Pacific including the Japan Sea. In Tateyama, central Japan, there was a sudden increase in the *S. mirabilis* population in 2013-2015, with the vast majority of individuals recognized as juveniles. Patterns of recruitment and growth were investigated by quantitative sampling of juveniles at four sites with different substrates/depths. Species identity of juveniles was determined through analysis of cytochrome oxidase subunit I (COI) gene sequences. Body sizes of juveniles ranged from 1.3 to 15.9 mm with those over 8 mm being collected only at the deepest collection site (15 m). Juveniles showed a unimodal size distribution throughout a year with no change in their median size. However, the population of juveniles seemed to consist of three cohorts. In addition, the size-frequency distributions showed various recruitment patterns depending on sites and season, indicating a possibility of migration from shallow to deep sites with their growth. Overall, our data demonstrate spatio-temporal 'patchiness' in the recruitment of *S. mirabilis* juveniles.

**Keywords**: population dynamics, DNA sequencing, size structure, *Scaphechinus mirabilis*

## Introduction

*Scaphechinus mirabilis* A. Agassiz is a sand dollar species that inhabits the intertidal and subtidal sandy substrates of northwest Pacific including the Japan Sea. The adults of *S. mirabilis* are known to form dense beds for several decades over their life span (Takeda 2008). Sand dollar larvae have a planktonic mode of life that lasts about one month (i.e. from fertilization to metamorphosis) under appropriate temperature and salinity conditions (Dautov & Kashenko 2008). The adults facilitate settlement and metamorphosis of larvae, but may increase juvenile mortality by disturbing substrates and through competition for food (Takeda 2008). Previous studies have indicated that juveniles tend to occur in habitats where adult density is relatively low.

In Tateyama, central Japan, there has recently been a sudden shift in sand dollar fauna from *Peronella japonica* Mortensen, a previously common species, to *S. mirabilis* with virtual disappearance of the former.

As there is a paucity of information on the population characteristics of *S. mirabilis*, occurrence of juveniles in Tateyama has provided a good opportunity for investigating its population structures. In this study, we aimed to analyse size-frequency patterns of the S. mirabilis population through quantitative sampling.

## Materials and Methods

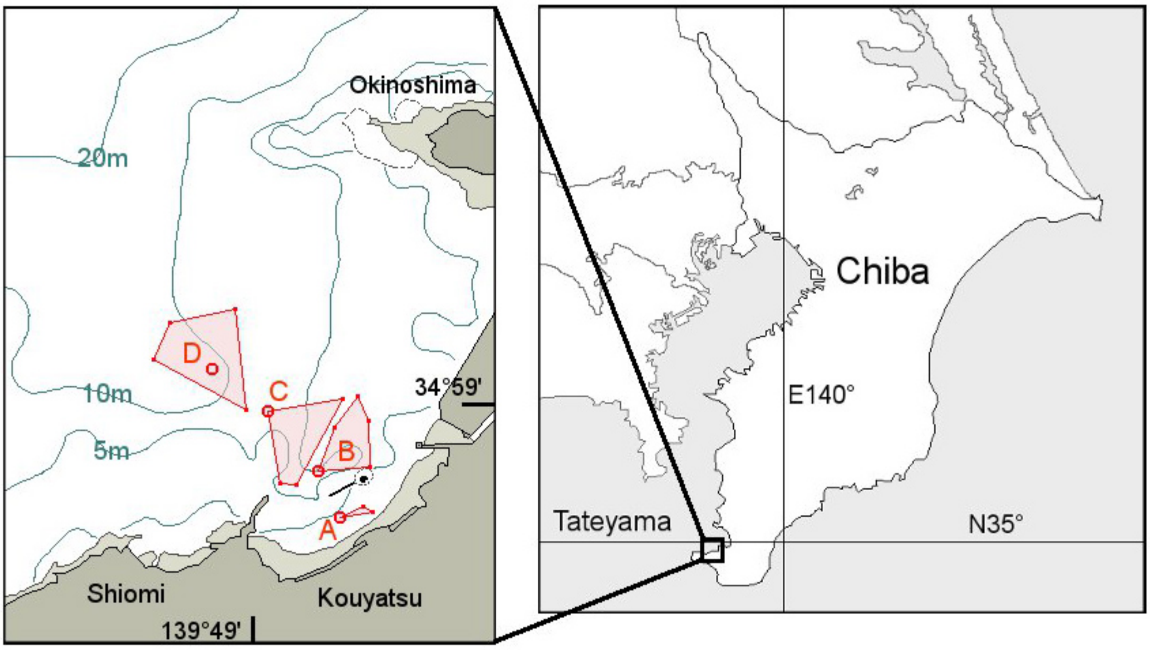
Sample collection

Dredge survey was conducted on a monthly basis between April 2014-March 2015 at four sites on the coast of Tateyama, central Japan. The four sites (Fig. 1) varied in depth (2-12m), substrate characteristics and temperature. Dredging was conducted from one coordinate of each collection site towards a constant direction for circa 120~210 m. We collected 10L of sand at each site. When the amount of sand was less than 10L, dredge sampling was repeated from the defined coordinate to get a sufficient quantity of sand. However, at site A, only 8, 6 and 5L of sand were obtained in October, November and December 2014, respectively, and less than 1L of sand in February and March 2015 despite 2-3 trials. Excess sand over 10L was thrown away. On return to the laboratory, sand was sieved with 1-mm mesh and organisms and particles collected were moved to a container (64×38×16 cm) filled with sea water. Sand dollar juveniles were collected by naked eye within two days of dredge sampling.

DNA extraction and sequencing

Three types of juveniles with different appearances occurred in the collection sites (Fig. 2). For species identification, we conducted sequence analysis based on cytochrome oxidase subunit I (COI) gene of 700 base pairs using the adults of sand dollar species, *Scaphechinus mirabilis* A. Agassiz, *Peronella japonica* Mortensen, *Clypeaster japonicus* Döderlein and *Astriclypeus manni* Verrill, and three types of juveniles collected in Tateyama, each represented by one individual (Table 1). A piece of mouth of *S. mirabilis* and *P. japonica* and juvenile body were immersed in CHAOS solution (modified from Fukami *et al*. 2004 -- guanidine thiocyanate, 4M; salkosyl, 4%; Tris-HCl (pH 8.0), 25ml; 2-mercaptoethanol, 0.1M) at 4ºC for 10 days. DNA was extracted from the solution using the DNeasy Tissue Kit (Qiagen K.K., Tokyo, Japan) following the manufacturer's protocol. The mitochondrial COI gene was amplified using EmeraldAmp PCR Master Mix (TAKARA BIO INC., Shiga, Japan) and generic primers for echinoderms according to the following protocol: 94 ºC for one minute, with 35 cycles at 98 ºC for 10 seconds, 50 ºC for 30 seconds and 72 ºC for one minute 30 seconds, and 72 ºC for 5 minutes. The amplification rate of the adult's DNA was low, so we added 1 M betarin to each solution before PCR reaction. PCR products were treated with ExoSAP-IT (GE Healthcare) before sequencing reactions. Sequencing reactions were performed by Operon Bio Technology (Tokyo, Japan) using the BigDye(r) Terminator v3.1 Cycle Sequencing Kit and the ABI 3730XL DNA Analyzer. The determined sequences were analyzed using MEGA6 (Tamura *et al*. 2013). The sequences data of *C. japonicus* and *A. manni* were obtained from the DNA Data Bank of Japan (DDBJ). Alignments were performed using MUSCLE (Edgar 2004), and these were checked by eyes and manually edited. Then, aligned DNA sequences of the partial COI gene were analyzed by the maximum likelihood method and phylogenetic analysis using a bootstrap test with 1000 resampling events. The DNA sequence data of the samples collected in this study were deposited at the DDBJ with the accession numbers shown in Table 1.

(a)




(b)

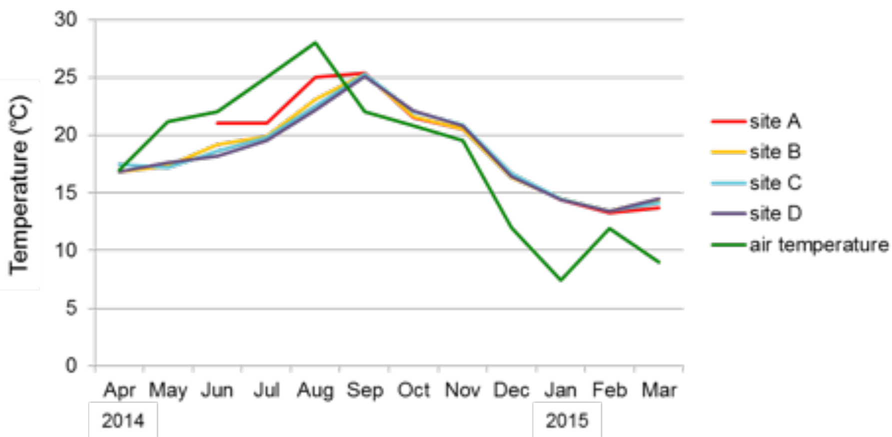



**Fig. 1** (a) Map of the study site in central Japan and the location of four sampling sites (in red). A net was hauled from the chosen coordinate of each site (○): site A, N 34°58'47.8" E 139°49'10.9", 1.0~2.9m depth, fine sand substrate; site B, N 34°58'53.5" E 139°49'08.1", 5.7~6.8m depth, sandy substrate; site C, N 34°58'58.8" E 139°49'02.2",5.0~7.7m, sandy substrate; site D, N 34°59'04.7" E 139°48'57.4", 8.9~15.0m depth, Mixture of sandy and rocky substrates.
(b) Water temperature at each collection site and air temperature. Water temperatures were measured using a logger during dredging. The air temperature data were derived from the Japan Weather association (http://www.tenki.jp/past/), referring to the time when dredge sampling was conducted.

## Measurements

Collected living juveniles were placed on a dish filled with sea water and the photographs were taken from the upper side using a video camera (JVCKENWOOD Corp., GZ-G5-B) set up on a stereo microscope (OLYMPUS, SZX7). Live individuals were recognized as those moving their spines or feet. The body sizes were measured as the length of the posterior-anterior axis via the centroid of five petals and the periproct of juveniles except for spines using ImageJ 1.47 (National Institutes of Health, Bethesda, Maryland, USA). After taking photographs, juveniles were preserved in 99.5% ethanol at -30 ºC.

## Statistical analysis

Water temperature and depth were measured during a dredging survey using a logger (HOBO Water Level U20-001-02, onset Computer Corp., USA, with HOBOware Pro version 3.7.1). The coordinate of each site was confirmed using GPS (eTrex Venture, GARMIN, USA).

Size frequency data were analyzed by the Kolmogorov-Smirnov test using R Studio version 0.98.1103. FiSAT II version 1.2.2 was used to construct size frequency distributions. February and March 2015 samples were taken with a 2-week interval only, so they were combined to make a larger sample (N=396) for cohort analysis.

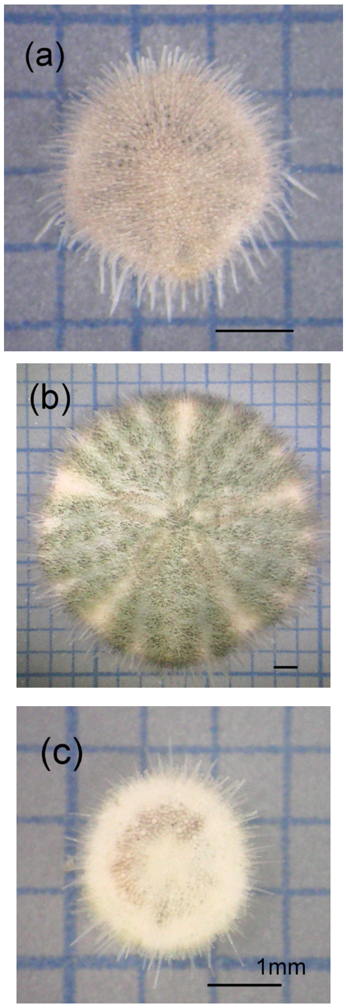



**Fig. 2** Juveniles of *Scaphechinus mirabilis* (a)-(b) and *Peronella japonica* (c) collected in this study. The scale bars are 1 mm.

**Table 1** List of sand dollar species used for sequence analyses in this study. Sequence data of *C. japonicus* and *A. manni* were obtained from DDBJ (Lee, 2011).

| Species | Stage | Collection site | Abbreviation | Accession number |
|---|---|---|---|---|
| *Scaphechinus mirabilis* A. Agassiz | juvenile | D | 1403 | LC096078 |
| *S. mirabilis* | juvenile | D | 1404 | LC096079 |
| *S. mirabilis* | adult | A | 1423 | LC096081 |
| *Peronella japonica* Mortensen | juvenile | C | 1401 | LC096077 |
| *P. japonica* | adult | D | 1421 | LC096080 |
| *Clypeaster japonicus* Döderlein | adult | Korea | *C. japonicus* | JQ341144 |
| *Astriclypeus manni* Verrill | adult | Korea | *A. manni* | JQ341142 |

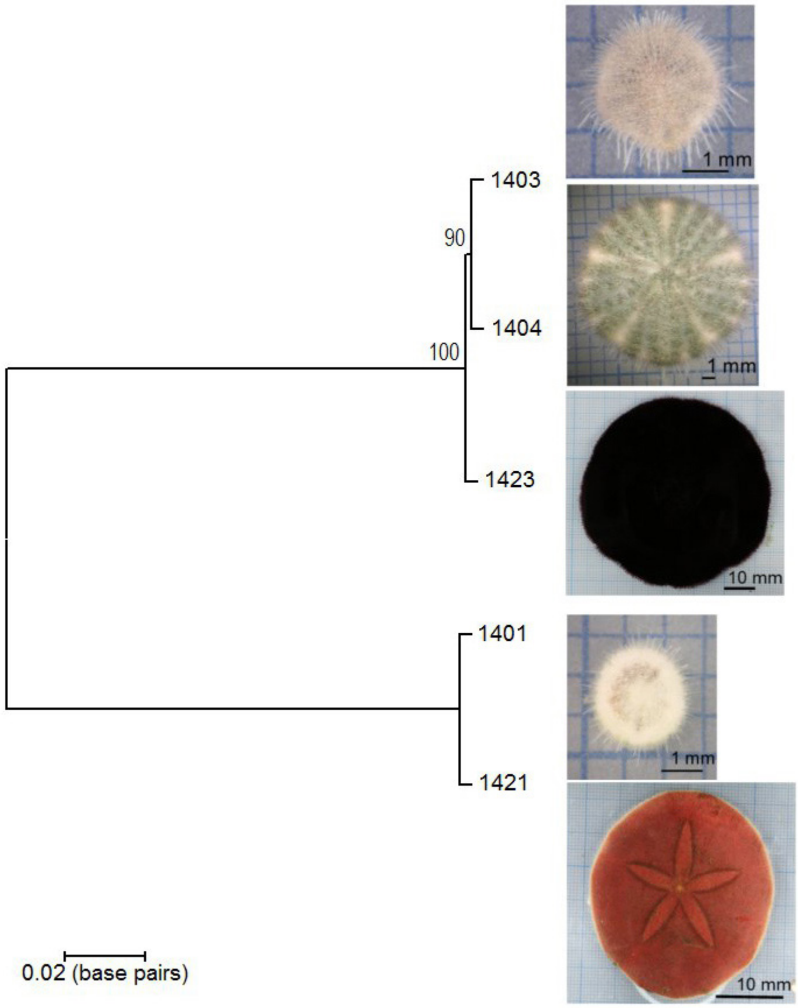



**Fig. 3** Consensus tree of sand dollar species based on cytochrome oxidase subunit I (COI) gene sequences. Phylogenetic analysis was conducted by the maximum likelihood method combined with a bootstrap test with 1000 resampling events.

## Results

### Species identification

Our sequence analysis revealed that two species of sand dollars occurred as juveniles in Tateyama, central Japan (Fig. 3). High bootstrap values were observed between the adult (designated as 1423 in Fig. 3) and the juvenile (1403 and 1404) of *S. mirabilis*, and for *P. japonica* (adult: 1421, juvenile: 1401), but less than 54% for interspecific comparisons (Fig. 3). This result shows the monophyletic nature of adults and juveniles, suggesting that two types of the three juveniles collected were *S. mirabilis* (Fig. 2).

### Size frequency distributions

Juveniles of *S. mirabillis* observed in this study had body sizes in the range of 1.3-15.9 mm. Adult individuals were not collected by dredging. Sample size varied with sites and months, with site B having very small sample sizes (an average of less than 4 individuals collected monthly) throughout the year. At site A, there were many juveniles from May to September, but few samples from December to March (Fig. 4). On the other hand, at site C and D, many juveniles were collected in February and March, but other months had few samples. The decline in number of collected juveniles in October may be due to heavy wave disturbances caused by two typhoons at a short interval.

The populations of juveniles showed a unimodal size distribution over a year and the size-frequency distributions showed various recruitment patterns. There were significant differences in size frequencies (Kolmogorov-Smirnov test, $p<0.05$) between two periods: May-June and July-August (Fig. 4a). In the case of site A, the distribution pattern of juveniles differed between May-Jun, July-August and January-February (Fig. 4b). At site D, a significant difference was only seen between February and March (Fig. 4d). At site C, there were no significant differences between all of adjacent months (Fig. 4c). Juveniles of over 8mm in size were collected only at site D from April to September.

An analysis involving curve fitting for the combined data

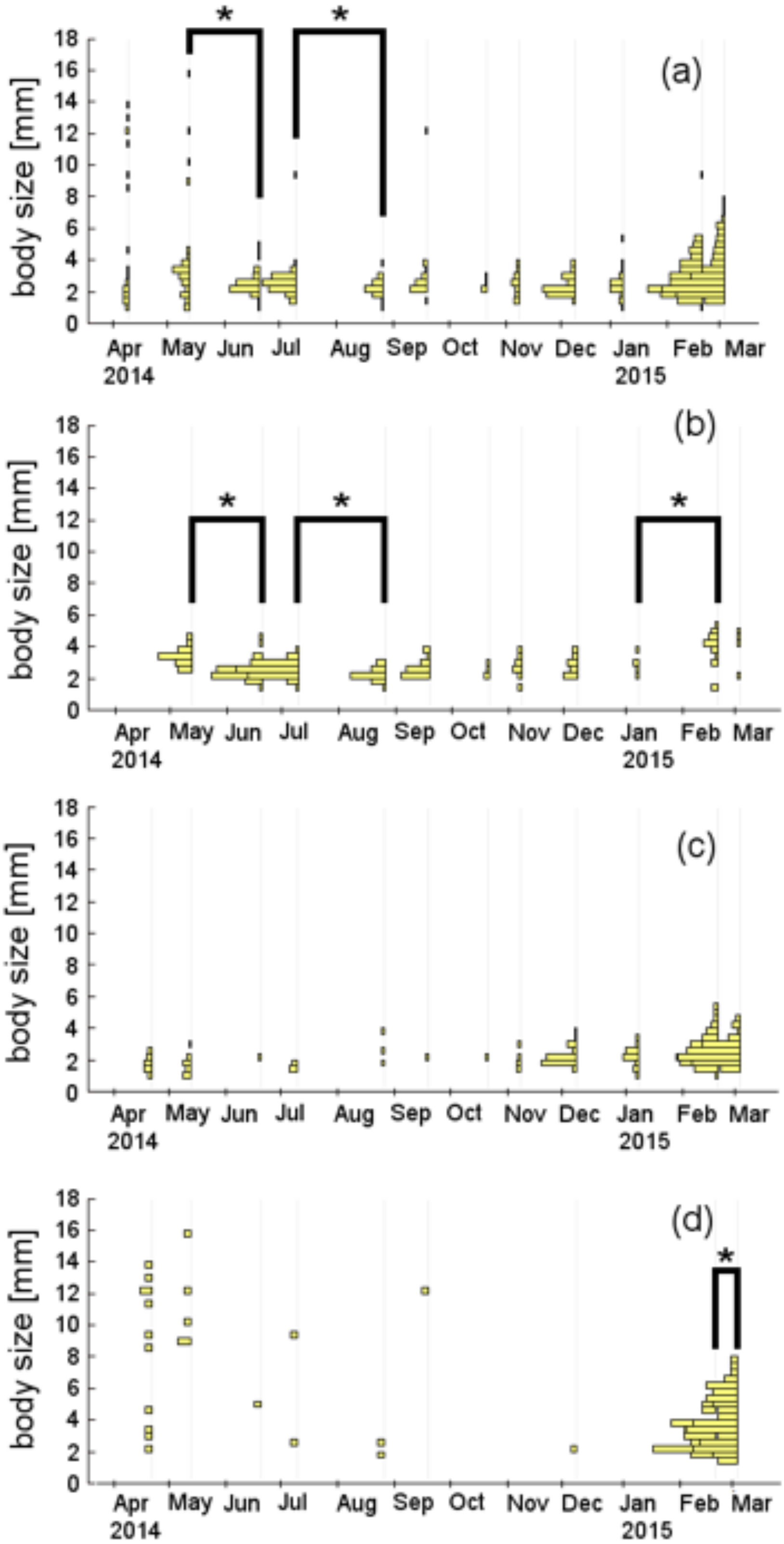



**Fig. 4** Size-frequency distributions of *S. mirabilis* juveniles collected from April 2014 to March 2015: (a) all sites combined, (b) site A, (c) site C and (d) site D. Data from site B were not shown due to small sample sizes. No April data for site A. The black lines and * indicate significant differences between the adjacent months (Kolmogorov-Smirnov test, $p < 0.05$).

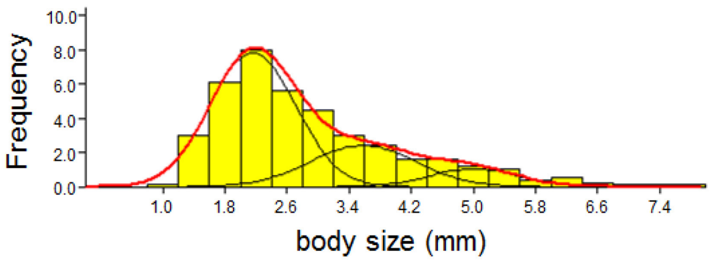



**Fig. 5** Multiple normal distributions fitted to the size-frequency data (N=396, February-March 2015) of *S. mirabilis*.

of February and March showed that the juvenile population consisted of three cohorts with mean body sizes of 2.2 mm, 3.6 mm and 5.0 mm (Fig. 5).

## Discussion

The size structure of juveniles showed little change through a year (Fig. 4a). The statistical differences shown in the size-frequency distribution of the whole data may be influenced by the occurrence of large individuals collected at site D. Size-frequency distributions were more variable at site A than at other sites (Fig. 4b-d), probably due to site A being more exposed and hence variable in environmental conditions.

In addition, we found the adults of *S. mirabilis* at site A, indicating the recruitment of new individuals into the population at this site. The breeding season of S. mirabilis is from October to November in Aomori, northern Japan (Takeda, 2008) and Okayama, south-western Japan. Of 10 *S. mirabilis* adults collected at site A in early December, five individuals had mature gonads (sperms and eggs were released after KCl injection). The fertilization rate was about 80~90% and fertilized eggs normally developed to become pluteus larvae. Planktonic larvae with adult rudiments metamorphosed in the aquarium with sand collected at site A. It took about one month from fertilization to metamorphosis in the laboratory at c. 20ºC. A previous study showed that spawning in *S. mirabilis* occurred when surface water temperature increased to 18 ºC (Kashenko 2009). In Tateyama, water temperature exceeds 18 ºC around May and Jun (Fig. 1b). However, the adults of *S. mirabilis* collected at the same site in May did not have mature gonads. These indicate that the reproduction of *S. mirabilis* in Tateyama is expected around November to December and recruitment may occur after December. Further, larvae could completely metamorphose in 28 days after the formation of adult rudiments, so they are thought to be able to settle and metamorphose after a long drifting.

Temporally contrasting patterns were seen at site A and at other two sites, site C and D. While site A had many juveniles from May to September, at site C and D, the number of juveniles suddenly increased in February and March. Moreover, the larger juveniles over 8 mm were collected only at site D from April to September. These results indicate the possibility of migration of juveniles with their growth from shallower to deeper sites. Juveniles may select more suitable microenvironments for their growth, depending on their growth stages and/or body size. The same tendency was revealed in the juveniles of *Echinarachnius parma* Lamarck: individuals < 28 mm inhabit deeper sites and those ≥ 28 mm inhabit shallower sites (Cabanac & Himmelman 1996).

Although many juveniles of *P. japonica* were observed in the same study site in 2012, few juveniles (and adults) were found in 2014-2015 and instead *S. mirabilis* juveniles were common. Decline in the number of *P. japonica* juveniles may be related to a sudden increase in a *S. mirabilis* population. However, dredging conducted at c.30 m depth yielded many juveniles of *P. japonica* but none of *S. mirabilis*, indicating a change in dominance from *P. japonica* to *S. mirabilis* and the possible movement of *P. japonica* juveniles.

In conclusion, our study indicated spatio-temporal variation in recruitment and distribution and a possibility of growth-related migration of juveniles of S. mirabilis on a Pacific coast of central Japan. A continuous research on their population dynamics is necessary to clarify their status and relations with other sand dollar species.

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## References

Cabanac A & Himmelman JH (1996) Population structure of the sand dollar Echinarachnius parma in the subtidal zone of the northern Gulf of St. Lawrence, eastern Canada. Canadian Journal of Zoology, 74, 698–709.

Dautov SS & Kashenko SD (2008) Development of the Sand Dollar Scaphechinus mirabilis. Russian Journal of Marine Biology, 34(6), 415–420. doi:10.1134/S1063074008060114

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research, 32(5), 1792–1797. doi:10.1093/nar/gkh340

Fukami H, Budd AF, Paulay G, Solé-Cava A, Allen Chen C, Iwao K & Knowlton N (2004). Conventional taxonomy obscures deep divergence between Pacific and Atlantic corals. Nature, 427, 832–835. doi:10.1038/nature02339

Kashenko SD (2009) Effects of Extreme Changes of Sea Water Temperature and Salinity on the Development of the Sand Dollar Scaphechinus mirabilis. Russian Journal of Marine Biology, 35(5), 422–430. doi:10.1134/S1063074009050083

Lee T (2011) A systematic study of Korean echinoids based on morphology and molecular phylogeny. Thesis Sahmyook University, Seoul, South Korea

Takeda S (2008) Mechanism maintaining dense beds of the sand dollar Scaphechinus mirabilis in northern Japan. Journal of Experimental Marine Biology and Ecology, 363, 21–27. doi:10.1016/j.jembe.2008.06.010

Tamura K, Stecher G, Peterson D, Filipski A & Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Molecular Biology and Evolution, 30(12), 2725–2729. doi:10.1093/molbev/mst197

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