Breeding season and life history of *Lingula anatina* after settlement in Amami-Oshima Island, Kagoshima, Japan

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**Abstract:** *Lingula* species are brachiopods inhabiting the tidal flats. In Japan, they are threatened by the marine pollution and decreasing habitat. Although these brachiopods are endangered in Japan, little is known about their life history. The aim of this study was to examine the life history characteristics of *Lingula anatina* after settlement in Kasari Bay, Amami-Oshima Island, Kagoshima, Japan, by measuring the gonad index and seasonal changes in size distribution. The results revealed that the breeding season of this species is from summer to early autumn, which is longer than that of other populations in Japan. Their settlement starts in winter after the planktonic stage, and they reach maturity after two or three years. Their longevity is more than four years and they breed every year. *Lingula* species are under taxonomic revision; therefore, for convenience, we named the species in Amami-Oshima as *Lingula anatina*.

**Key words:** Amami-Oshima, breeding season, *Lingula*, life history, tidal flats

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**Introduction**

Brachiopods of the genus *Lingula* have been regarded as living fossils because their morphology has not changed since the end of the Precambrian (Tazawa 2010). They are suspension feeders filtering organic materials by a pair of crowns (Sato 2000), and inhabit the lower area of intertidal or subtidal zones with sandy or muddy sediment by attaching their pedicles to the substrate (gravel or coral gravel) (Emig 1997). Most of their habitat is threatened by coastal development and environmental degradation in the coast of Japan (Japanese Association of Benthology 2012).

The taxonomy of the genus *Lingula* was reconstructed to include seven different species, with four Japanese species, *Lingula adamsi* Dall, 1873, *Lingula anatina* Lamarck, 1801, *Lingula reevei* Davidson, 1880 and *Lingula rostrum* Shaw, 1798 (Kuramochi et al. 2001). Japan Ministry of Environmental (2017) follows the classification proposed by Nishizawa et al. (2010) separating *L. anatina* into four species, *Lingula nipponica* Hayasaka, 1931 (Mutsu Bay, Aomori Prefecture in northern Japan), *Lingula* sp. 1 (Amami-Oshima Island of Nsne Islands), *Lingula* sp. 2 (Ariake Bay in Kyushu), and *Lingula* sp. 3 (Izu Shimoda, Shizuoka Prefecture in central Japan). However, the Japanese Association of Benthology (2012) has described Japanese “Midorishamisenngai” in the coast of Japan as *L. anatina*.

Besides the above taxonomical controversy, information about their life history traits, including maturation time, breeding season, and growth after settlement is necessary for the conservation of their populations. Although the population structure and life history of *L. anatina* including its reproductive ecology, have been studied in Mutsu Bay (Akuta 1995) and Ariake Bay (Ito 2017a, b), little is known about the populations in Amami-Oshima. Therefore, the primary aim of this study was to clarify the breeding season and life history of the *L. anatina* population in Amami-Oshima by measuring their gonad index and seasonal changes in size distribution.

As a matter of practical convenience, we named the species of *Lingula* in Amami-Oshima (*Lingula* sp. 1 in Japan Ministry of Environmental 2017) as *L. anatina*; and *Lingula* sp. 2 and *L. nipponica* as *L. anatina* in the Ariake Bay and Mutsu Bay, respectively. Although Kuramochi et al.
R. Fujii et al. (2012) concluded that the species of *Lingula* in the Ariake Bay belongs to *L. reevei* based on their shell morphology, we herein refer to the classification of Itoh (2017b).

**Materials and Methods**

This study was conducted in the Tekebu tidal flats located inner part of Kasari Bay in northern Amami-Oshima (28°26′28.1″N, 129°39′56.3″E) (Fig. 1). The study site was located in the western area of the Tekebu River, the aggregations of *Lingula anatina* have been found in a previous study (Ogata et al. 2017). The bottom sediment of the survey site was composed of sandy mud and cobbles at shallow (<10 cm) and deeper depths (>10 cm), respectively. Individuals of this species appear to settle on small gravels at shallow depths (<10 cm) and on cobbles deeper depths (>10 cm) (Ogata et al. 2017). Given that this species is specified as “rare wild species”, sampling of these individuals is prohibited by the Amami City. Therefore, we released all specimens in the field survey after measurements. Specimens collected for measuring the gonad index were captured after obtaining permission from the Amami City.

Field survey was carried out almost monthly from May 2015 to October 2016 and from February to October 2017. A 20 m × 20 m study area was set up at 65 cm under the mean tidal level. Ten quadrats (15 cm × 15 cm) were randomly distributed throughout the study area in every survey. The sediment in each quadrat was sampled at the depth of 10 cm and sieved using a 2-mm mesh. After collecting the specimens of *L. anatina* in the sieve, we sampled located at deeper depths (10–30 cm) by hand. Since the number of cobbles in the sediment increased for deeper (203 cm), it is possible that the number of brachiopods also decreased at deeper depth (>30 cm). Moreover, the large numbers of cobbles at deeper depths (>30 cm) compromised sampling, which was difficult to sample them without injury. Therefore, sampling was conducted at a maximum depth range of 30 cm.

The temperature of the seawater was measured inside holes left in the sediment after sampling using a digital thermometer, CT-460WR (CUSTOM corp.). These measurements were conducted mainly in the middle of the day. After measuring the shell lengths using a digital caliper, CD67-S15PM (Mitutoyo corp.), we released all specimens, as this species is specified as “rare wild species”. To calculate the modal value of shell length in the size frequency distribution, we used the mclust package in R3.3.2 (Fraley & Raftery 2002). The best model fitting cohort number and homoscedasticity among cohorts was chosen for each month using the BIC criterion (Schwarz's Bayesian Information Criterion).

To analyze seasonal changes in gonad index, we collected monthly (March-November 2017) around 20 specimens of *L. anatina* showing larger shell lengths than 25 mm. After removing the dorsal shell, the wet weight of the soft tissue and gonads were weighed using a digital weighing scale, HR202i (A and D corp.). The gonad index of each sample was calculated (gonad wet weight/weight without dorsal shell).

**Results**

**Population characteristic after settlement of *Lingula anatina***

The density of *Lingula anatina* in the tidal flats of Tekebu ranged from 411.2 ind. m⁻² to 57.7 ind. m⁻² during the study period. The density increased from spring to summer in 2015 and 2016, with more than 400 ind. m⁻² found...
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in July, and decreased throughout autumn in 2015. For both years, the number dropped to fewer than 200 ind. m$^{-2}$ during the rainy seasons in June 2015 and May 2016, ranging from 200 to 310 ind. m$^{-2}$ in 2017 (Fig. 2).

Shell length (SL) of specimens ranged from 2.5 to 42.8 mm. Individuals were separated into six cohorts based on their shell sizes (Fig. 3). Specimens with smaller than 10.0 mm appeared in June and August of 2015, from March to October of 2016; and from April to June and August of 2017. Shell lengths increased to more than 10.0 mm in September in the years of 2015 and 2017.

The mean shell size of each cohort was calculated by using the mclust package in R3.3.2 (Fig. 4). The youngest cohort (Cohort VI), which appeared in April 2017 with a mean shell length of 6.9 mm and it increased to 12.5 mm in June 2017. The second youngest cohort (Cohort V) appeared in March 2016 with a mean shell length of 5.2 mm. However, this cohort disappeared from February to June 2017. Cohort V shifted rapidly to a mean shell length of 19.0 mm in October 2017. The third youngest cohort (Cohort IV) appeared in late August 2015. This cohort shifted to a mean shell length of 28.6 mm in August 2016. After sifting a mean shell length of 25 mm in September 2016, the growth rate dropped to very low values. The third oldest cohort (Cohort III) was found at the beginning of the study (May 2015) and shifted from an initial size of 17.3 mm to 31.8 mm over one year. After sifting a mean shell length of 30 mm in June 2016, the growth rate dropped to very low values. The second oldest cohort (Cohort II) also was also found in the beginning of the study with an initial shell length of 26.6 mm (May 2015), but shifted slowly to a shell length of 28 mm. This cohort disappeared in March 2016. The oldest cohort reached 35 mm in July 2015 and disappeared in October 2015.

Seasonal change of seawater temperature and gonad index

The monthly average (N=10) seawater temperature in the sediment ranged from 18.8°C to 32.6°C from March to November 2017 (Fig. 5). The temperature increased from March to September reaching almost 32°C in June and dropped to 22.7°C in November after reaching its highest mean value of 32.6°C in September.

The gonad index was measured every month for 20 individuals with larger shells than 25 mm. Individuals with smaller shells than 28 mm showed no gonadal development. For such specimens, it was difficult to distinguish between males and females. Therefore, the sex and gonad index was determined only for the specimens with larger shell lengths than 28 mm (Fig. 5). Individuals with milky white gonads were classified as males, while those with brown gonads were classified as females. The mean value of the gonad index was 1.64% in males and 1.68% in females for April. The gonad index increased until the autumn, reaching 8.35% in August and 7.76% in September in females and males, respectively. It was greater than 5.50% between August and October 2017.

Discussion

Seasonal changes in the density and size distribution of individuals of *Lingula anatina* in Amami-Oshima were investigated in the Tekebu tidal flat, from May 2015 to October 2017. The density increased from the spring to the summer in 2015 and 2016 (Fig. 2). This increase seemed to be caused by the presence of a new cohort, since the smallest size class (2–3 mm in shell length) appeared in March 2016 (Fig. 3). The presence of such size classes might be explained by growth of newly settled juveniles to larger shell lengths than 2 mm, or by immigration of young individuals of that size range. Although no obvious increase was found in density in the summer of 2017, the smallest size class was recorded in April (Fig. 3). It is possible that the abundance of individuals settling or the surviving after settlement were lower than in the previous two years.

The density dropped in the middle of the rainy season (Fig. 2). Death or emigration of individuals to areas outside of the survey area could be a probable cause explaining such results. However, as dead shells were not found in the survey area and given that the density recovered in the next month (Fig. 3), such explanations seem unlikely. In addition, strong rains in this season could also compromise detection of the individuals in the survey area. However, this explanation seems also unlikely, as small sized individuals we detected in those months (Fig. 3). Another possibility is that brachiopods buried themselves at deeper depths than 30 cm in the sediment to avoid changes in salinity caused by the rain. This explanation is supported by the lack of larger individuals in the size histogram for these months and by a previous study showing that large
Fig. 3. Seasonal change in the size-distribution of *Lingula anatina* from May 2015 to October 2016 and from February 2017 to October 2017. n refers to the number of samples.
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individuals can reach deeper depths (Fujii 2016). In addition, we did not find such decreases in density in June of 2017, when the amount of rainfall in the rainy season was substantially lower, with 86% of usual years against 141% and 103% in 2015 and 2016, respectively (Japan Meteorological Agency online).

In the present study, the gonad index of individuals of *L. anatina* in Amami-Oshima showed a peak from August to October (Fig. 5). A previous study reported that the larvae of *L. anatina* in Misaki, central Japan started settling when reaching a shell length of 0.65 mm after completing their six-week planktonic stage (Yatsu 1902). Therefore, settlement of individuals of *L. anatina* in Amami-Oshima is expected to have occurred from autumn to winter.

The sampled individuals of *L. anatina* reached approximately 15 mm and 10 mm of shell length in autumn 2015 and 2016, respectively (almost one year after breeding). The growth rate of the individuals collected in this study was higher those of individuals of other regions in Japan. For example, *L. anatina* in Misaki reached 5 mm after settling for one year of central Japan (Yatsu 1902), and *L. anatina* in Ariake Bay (Kyushu) reached 10 mm after one year of breeding (Itoh 2017a). The growth rate of the individuals that settled in 2017 is expected to have been an intermediate value between previous two years, given their size distribution in the summer of 2017 (Fig. 4).

The minimum shell length at maturity of *L. anatina* in Amami-Oshima was 28 mm with individuals likely spending around two to three years of settlement before reaching such size range (Fig. 4). Itoh (2017a) showed that individuals of *L. anatina* in Ariake Bay mature two years after settling having a shell length of around 30 mm at maturity. Individuals of *L. anatina* in Mutsu Bay spend eight years reaching maturity (Akuta 1995), while individuals of *L. anatina* from northern Australia require three years to reach maturity (Kenchington & Hammond 1978).

A maximum of four cohorts were found during the same period of the study (Fig. 3), suggesting that their life span is longer than four years. However, as the size distribution much overlapped among cohorts, it is difficult to evaluate their exact life span. After reaching a mean shell length of around 30 mm, the growth rate abruptly decreased showing a maximum length around 40 mm. This result suggests that the cohort older than three years reached a similar shell length compared to those of the largest cohort. The smallest cohort could be clearly distinguished after 10 months of settlement, remaining undetected from winter to spring (Fig. 4). The lower number of individuals found in winter compared to all other seasons might be related to sampling artifacts caused by small sample sizes.

Itoh (2017b) reported that the life span of the individuals of *L. anatina* from Ariake Bay was of seven years further suggesting that their longevity might exceed ten years. Our data showed that *L. anatina* in Amami-Oshima shared similar life history traits as the individuals of Ariake Bay, including age and size at maturity, and initial growth rate. Therefore, it is expected that the life span of *L. anatina* in Amami-Oshima resembles those of individuals in Ariake Bay.

Individuals of *L. anatina* in Misaki, central Japan were reproductively active only during mid-summer (July and August) (Yatsu 1902, Kume 1956), while the individuals found in Ariake Bay showed an increase in the gonad index in May with a peak in July (Ito 2017a). The breeding season of the latter individuals began two months earlier than those of the individuals in Misaki, central Japan. The gonad index of individuals of *L. anatina* in Amami-Oshima increased in June and August in females and males, respectively. This result suggests that the peak of reproduction of *L. anatina* in Amami-Oshima occurs later than those of *L. anatina* in Ariake Bay and in Misaki, with individuals of Amami-Oshima showing a peak between...
August to October. Akuta (1995) has indicated that the gonad index of individuals of *L. anatina* in Mutsu Bay of Aomori was regulated by water temperature (Fig. 18 in Akuta 1995), with reproduction finishing in the end of August. Figure 6 shows the seasonal changes of seawater temperature in some areas of studies referred here. The reproduction of *L. anatina* in Aomori and Misaki, seemed to complete in the months that coincide with highest seasonal temperature. In Singapore, however, the larvae of *L. anatina* appeared during all seasons, suggesting that reproduction occurs throughout the year. The seawater temperature remains at approximately 28°C throughout the entire year in this region (Chaung 1959). Such results confirm that the breeding of *L. anatina* is regulated by the temperature of seawater. While the gonad index of individuals of *L. anatina* in Amami-Oshima increased with increasing seawater (Fig. 5), the reproduction continued even after temperature dropped. In contrast, the reproduction of individuals in Ariake Bay completed in July (Itoh 2017a), when the seawater temperature was still increasing (Fig. 6).

The life cycle of *L. anatina* in Amami-Oshima can be described as follows: breeding occurs from summer to early autumn, with larvae settling on the sediment of tidal flats from autumn to winter. Two to three years later, *L. anatina* reaches maturity and reproduce annually. The reproductive season is longer than in individuals of other areas in Japan and continues across the highest temperature season. Such a long reproductive season as reported in the present study may contribute to the conservation of the populations in Amami-Oshima. Importantly, we focused our discussion on intraspecific differences in the life history traits of local populations. However, considering the taxonomic controversy regarding the species of the genus *Lingula*, such differences might be inter-specific. Further taxonomical study is needed to clarify the life history of each population of *Lingula* for the conservation of these species.

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| Table 1. Comparison of breeding seasons and seawater temperature among regions. |
|---------------------------------|-----------------|-----------------|
| **Regions** | **Sea water temperature** | **Breeding season** |
| Amami-Oshima (this study) | 18.8°C (March) to 32.6°C (September) | from August to October |
| Misaki (Central Japan) | 13°C (March) to 27°C (August) | July and August |
| (Yatsu 1902) | | |
| Townsville (northern Australia) | 22°C (July) to 31°C (January) | from November to March |
| (Hammond 1982) | | |
| Northern Red sea | 26°C to 28°C | from March to September |
| (Ashworth 1915) | | |
| Singapore | over 28°C | all season |
| (Chaung 1959) | | |
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