Reproductive season and life span of an estuarine polychaete, *Simplisetia erythraeensis* (Annelida, Nereididae), in Southern Japan

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**Abstract:** The reproductive season and life span of the common estuarine polychaete *Simplisetia erythraeensis* (Annelida: Nereididae) was investigated by monthly sampling during the years 2015 and 2016 in two tidal flats at two different regions in southern Japan: Shigetomi in Kagoshima Bay, Kyusyu Island, a warm-temperate region and Ichi on Amami-Oshima Island, Ryukyu Islands, a maritime subtropical region. At both sites, one or two cohorts were distinguished simultaneously using monthly body-width histograms. Recruitment of new cohorts occurred from August in Kagoshima Bay and from July on Amami-Oshima Island. Ovigerous females were observed from March to August in the former area and from April to July in the latter area, with the highest female maturity rate (number of females with oocytes/total number of adults larger than the smallest mature size) in July in both areas. The maximum value of the mean oocyte size of each female increased with increasing female maturity rates. The density of each cohort decreased from May onward because of the death of adults after reproduction, which resulted in the complete disappearance of the cohort in early October. Our results showed that the life span of *S. erythraeensis* was one year, and the reproductive period occurred in summer.

**Key words:** Amami-Oshima, Kyusyu, life history, population structure, reproductive season

**Introduction**

Polychaetes are common marine organisms and frequently dominate the macrobenthic fauna of estuaries and tidal flats (Mettam 1981, Sato 2006, 2017). Polychaetes mediate the environment of tidal flats by decomposing organic matter in the sediment through feeding and digesting (Kurihara 1983, Kumagai & Kurihara 1989) and turning over the sediment by digging (Sayama & Kurihara 1983, Kikuchi 1987). Therefore, better understanding of the ecology of polychaetes including detailed information on their life histories and reproduction is needed to improve the management of tidal flats and to conserve the functioning of these ecosystems (Grassle & Grassle 1974, Connell & Slatyer 1977, Pearson & Rosenberg 1978). Population dynamics and life history traits have been intensively studied in some species of Capitellidae (Tsutsumi & Kikuchi 1984, Tsutsumi 1987, 1990) and Spionidae (Levin 1984, Levin et al. 1991, Yokoyama 1990), which are considered bioindicators of organic pollution. Several studies investigated reproduction of nereidid species and observed that reproduction in numerous species is affected by temperature (Levin & Creed 1986, Olive 1995, Olive et al. 1997). However, little is known about the life history of most species of Nereididae.

*Simplisetia erythraeensis* (Fauvel 1918) is a nereidid species with a wide geographical distribution including Madagascar (Fauvel 1918, Day 1967), Mozambique (Day 1967), South Africa (Day & Morgans 1956), the Red Sea (Fishelson 1971, Vine 1986), and eastern Asia (Sato 2017). This species is common in estuarine tidal flats of Japan from Hokkaido to the Ryukyu Islands (Sato 2017); in a large survey of macrobenthic organisms in tidal flats of 157 sites throughout Japan from 2002 to 2004, *S. erythraeensis* was found at 69 sites, from Aomori Prefecture.

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located at the northern tip of the main island (40°56′N, 140°58′E) to Iriomote Island which is located in the southwest (24°18′N, 123°54′E) (Iijima 2007). This species likely plays an important ecological role in tidal flats ecosystems in Japanese estuaries where it is highly abundant.

Kalejta (1992) examined the population dynamics of *S. erythraeensis* in South Africa; however, no corresponding studies on life histories and seasonal variations in population size or reproductive patterns of this species were conducted in Asia. Therefore, we conducted the research on life history of *S. erythraeensis*, particularly regarding population dynamics in the benthic stage and the reproductive period in tidal flats at two sites in southern Japan. Each site belonged to a different climatic region: Kagoshima Bay was a warm temperate region and Amami-Oshima Island as part of the Ryukyu Islands was considered a maritime subtropical region. The ecological characteristics of *S. erythraeensis* were thus compared between two different climatic regions.

### Materials and Methods

#### Study sites

Field surveys were conducted at tidal flats at Shigetomi in Kagoshima Bay in southern Kyushu Island and at tidal flats at Ichi on Amami-Oshima Island in the Ryukyu Islands (Fig. 1), representing two different climatic regions.

The tidal flats at Shigetomi (31°42′26.3″N, 130°37′18.8″E) are located at the estuary of Omoi River, in the inner portion of Kagoshima Bay in a warm temperate area; the average air temperature at this site was 18.8°C, and the annual rainfall was 3,664 mm in 2015 (Japan Meteorological Agency online). The mean sea level was 155 cm above the datum line. This sampling site was selected because of the high population density of *Simplisetia erythraeensis* observed in previous studies (Yamamoto et al. 2009, Hayashi & Yamamoto 2011).

The tidal flats at Ichi (28°13′35.6″N, 129°26′53.7″E) are located in the estuary of Sumiyo Bay on the eastern side of Amami-Oshima Island, which was considered a maritime subtropical region; the average air temperature at this site was 22.1°C, and the annual rainfall was 2,642 mm in 2015 (Japan Meteorological Agency online). The mean sea level was 119 cm above the datum line. Some small mangrove trees were growing in the supratidal zone of this site. This sampling site was selected because of the high population density of *S. erythraeensis* observed in a previous study (Ogata et al. 2017).

#### Sample collection

Specimens of *Simplisetia erythraeensis* were collected during the lowest tide of each monthly spring tide during the period from September 2015 to October 2016 at Shigetomi in Kagoshima Bay and from April 2015 to October 2016 at Ichi on Amami-Oshima Island. At each study site, a survey area of 100 m² was made; 5×20 m at Kagoshima Bay and 10×10 m on Amami-Oshima Island. Five quadrats (15×15 cm) were established in randomly chosen locations in each survey area, every month. In each quadrat, sediment was excavated to a depth of 10 cm using a hand shovel and was sieved through a 0.5 mm mesh and a 1 mm mesh. All polychaetes were collected by hand using tweezers and were fixed in 10% formalin.

#### Sediment temperature

HOBO Pendant data loggers (HOBO, Inc.) were used to measure sediment temperature in the habitat of *Simplisetia erythraeensis* at both study sites. When a primary logger failed or could not be retrieved, backup loggers were used. The sets of loggers were buried at each site at a depth of 5 cm, and sediment temperature was recorded every 30 min. The loggers were retrieved at each monthly sampling for data processing.

#### Seasonal changes of body size distribution

Population density was calculated by counting the number of individuals collected with each of the both sieves (0.5 mm and 1 mm mesh). The body size was defined by body width (BW) rather than by body length (BL) as polychaetes are frequently torn apart during sieving. The maximum body width excluding parapodia on the anterior chaetigers 10–20 was measured in all collected specimens of *Simplisetia erythraeensis*. All measurements were made with an accuracy of 25 μm using a stereomicroscope with four-fold magnification. For complete specimens, BL was also measured in order to develop regression equations of BW and BL. BW can be converted to BL for each site based on the high degree of significance of the regression equations as follows: BL (μm)=47.50 BW (μm)–4159.7 ($r^2=0.791$, n=120, $p<0.001$) at Shigetomi in Kagoshima Bay; and BL (μm)=50.03 BW (μm)–2704.8 ($r^2=0.58$, n=70, $p<0.001$) at Ichi on Amami-Oshima Island. Based
on these equations, we used BW as body size indicator.

Sexual maturity and size of oocytes

For every preserved specimen, the dorsum around chaetiger 50 was dissected on a glass slide to remove the coelomic gametes in order to examine sexual maturity. The size of oocytes does not differ between different parts of the body (Ueno et al. in preparation); we systematically examined the proximity of chaetiger 50. Specimens with oocytes or spermatocytes were considered mature females or males, respectively. The number of specimens of each sex was recorded. The minimum BW of mature females and males was 700 µm (see Results section); therefore, the maturity rate was defined and calculated as the proportion of the number of mature females or males to the number of individuals with a BW above 700 µm. Of each female, 20–30 randomly chosen oocytes were photographed using optical microscope with 20X objective lens, and the diameter of oocytes was measured using ImageJ 1.46r software (National Institutes of Health online).

Statistical analysis

Size-frequency histograms were produced for each population. The data were used to identify cohorts and to investigate the recruitment and growth of each cohort. From the size-frequency distributions, cohorts were distinguished using the R 3.3.1 package mclust 5.2 (Fraley & Raftery 2002).

Results

Seasonal temperature changes in the sediment

Figure 2 shows seasonal variation of sediment temperature at both sites from November 2015 to October 2016. During this period, the temperature ranged from −0.5°C in January to 33.0°C in August at Shigetomi in Kagoshima Bay and from 7.4°C on January to 35.0°C in August at Ichi on Amami-Ohshima Island, with an average of 20.9°C and 24.1°C, respectively. The temperature was consistently higher at Ichi than at Shigetomi, and the differences between the two sites were largest during winter (December to March). The highest daily temperature exceeded 30°C in a period from the end of May to September on Amami-Ohshima Island, and from July to August in Kagoshima Bay.

Seasonal change of density and size distribution in Kagoshima Bay

A total of 1,372 specimens of *Simplisetia erythraeensis* were collected at Shigetomi tidal flats in Kagoshima Bay. During the study period from September 2015 to October 2016, the mean density was 1,006 ind. m⁻² (Fig. 3). The density increased from September 2015 to February 2016 and fluctuated substantially from March to June 2016. The highest mean density occurred in April 2016 (1,653±1,157 ind. m⁻²), whereas the lowest mean density was observed in July 2016 (587±168 ind. m⁻²).

Our cohort analysis using size-frequency data was based on BWs of samples collected from September 2015 to October 2016. From August to September 2016, two cohorts were distinguished (Fig. 4A): cohort i seemed to comprise

Fig. 2. Seasonal variation in sediment temperature. Shown are data from November 2015 to October 2016 at (A) Shigetomi (Kagoshima Bay) and (B) Ichi (Amami-Oshima Island). The highest and lowest temperatures of the day are shown as solid and dashed lines, respectively. Data from April 9 to April 22 in 2016 at Ichi is not shown due to the loss of the data logger because of a flushing event.

Fig. 3. Seasonal variation in the density (ind. m⁻²) of *Simplisetia erythraeensis* at Shigetomi (Kagoshima Bay) and Ichi (Amami-Oshima Island). Solid circle: Shigetomi (Kagoshima Bay). Open circle: Ichi (Amami-Oshima Island). Each plot represents a mean of five quadrats (mean±SD). The x-axis position of each plot corresponds to the sampling date.
individuals recruited in 2015, and cohort ii consisted of individuals likely recruited in 2016. A maximum BW of 1,200 μm was recorded in April 2016, whereas a minimum BW of 200 μm was recorded in September 2015, August 2016, and October 2016.

Figure 5A shows the seasonal changes in mean BW of each cohort. The mean BW of cohort i was 459 μm in September 2015 and gradually increased to 665 μm in February 2016 and to 750 μm in May 2016. The mean BW of cohort ii was approximately 300 μm in August 2016 and increased to almost 430 μm in September.

Seasonal change of density and body size distribution on Amami-Oshima Island

A total of 1,234 specimens of *Simplisetia erythraeensis* were collected at Ichi on Amami-Oshima Island. The mean density was 588 ind. m$^{-2}$ during the study period from April 2015 to October 2016 (Fig. 3). Population density increased from August to October 2015, gradually decreased later until July 2016, and increased again thereafter. In 2015, the highest density was observed in November (844±241 ind. m$^{-2}$), and the lowest density was recorded in August (98±73 ind. m$^{-2}$). In 2016, the highest density was observed in September (1,236±358 ind. m$^{-2}$) and the lowest density in July (169±80 ind. m$^{-2}$).

Three cohorts were distinguished during the study period from April 2015 to October 2016. Two distinct cohorts coexisted in the periods from August to September 2015 and from July to August 2016 (Fig. 4B). Cohorts I, II, and III seemed to comprise individuals recruited in 2014, 2015, and 2016, respectively. The maximum recorded BW was 1,200 μm, which was observed in May 2015; the minimum BW was 125 μm, recorded in September 2015.

Figure 5B shows seasonal changes in mean BW for each
cohort. The mean BW of cohort II remained relatively stable at around 400 µm in an early phase from August to December 2015, then increased to 857 µm in May 2016, and then decreased to 601 µm in August 2016. The mean BW of cohort III was approximately 340 µm in August 2016, which was less than that of cohort II at the same stage.

Seasonality of reproduction

During the monthly surveys, a total of 59 mature females and 19 mature males were collected from Shigetomi in Kagoshima Bay in 2016, and in 2015 and 2016, 42 and 17 females and 6 and 3 males, respectively, were collected from Ichi on Amami-Oshima Island. At both sites, the minimum BWs of the mature females and males were 700 µm (Fig. 6), and the largest BW of ovigerous females was 1,100 µm in Kagoshima Bay in April 2016 and 1,200 µm on Amami-Oshima Island in May 2015.

In Kagoshima Bay, mature females were observed from March to August 2016, and mature males from June to August 2016 (Fig. 7A). A ratio of mature females and males to individuals with a BW larger than 700 µm (a threshold defined by the smallest mature individuals) was used as an index for determining the reproductive period of *Simplisetia erythraeensis*, and was referred to as the maturity rate. The maturity rate in females began to increase from March (5%) to May (30%), and peaked in July (49%). After that, it decreased to 17% in August and was zero in September 2016. No mature females were found from September 2015 to February 2016. The maturity rate in males peaked at around 15% from June to August 2016.

On Amami-Oshima Island, mature females were collected in a period from April to July 2015 and from April to June 2016 (Fig. 7B). The female maturity rate increased from April (17%) to May (41%), showed a relatively high and constant level in June and July (28–37%), then rapidly decreased to zero in August, but increased again in July (37%) in 2015. No mature females or males were found from August 2015 to March 2016. Thereafter, the female maturity rate increased to 11% in April and peaked at 29% in June in 2016. Mature males occurred in June (10%) and July (8%) 2015 and in May (6%) and June (3%) 2016.

The largest oocyte diameters in Kagoshima Bay and Amami-Oshima Island were 150 µm and 138 µm, respectively, and the smallest ones were 12 µm and 10 µm, respectively. In Kagoshima Bay, the maximum value of the individual oocyte size mean was around 40 µm in April and increased to more than 100 µm from June to July (Fig. 8). The minimum value of the individual oocyte size means was consistently around 20 µm from March to August, and the difference in oocyte size between females increased from May to July.

On Amami-Oshima Island, the maximum value of the individual oocyte size means was around 40 µm in April and increased to more than 110 µm in July 2015. In 2016, it was above 80 µm from April to June.

Discussion

In the present study, the population characteristics of *Simplisetia erythraeensis* larger than about 200 µm BW at Shigetomi in Kagoshima Bay and at Ichi on Amami-Oshima Island were investigated on a monthly basis. Population densities showed seasonal changes at both sites with a rapid decrease from spring to summer and an increase from late summer to autumn (Fig. 3). The population at Kagoshima Bay showed larger density variation than that on Amami-Oshima Island, and this variation may be caused by spatial density variation in the surveyed area as the variance of density was considerably larger in Kagoshima Bay than Amami-Oshima Island (Fig. 3). The cohort analysis detected several annual cohorts at both study sites. Cohorts newly appeared in July (cohort III) and August (cohorts ii and II), after which their densities increased abruptly in the first two to three months after this, and they seemed to disappear in September in the following year (Fig. 4). The fact that only a single cohort existed in the period from the end of autumn to early summer suggests that each cohort lives for only one year. We will discuss about
Juveniles of the smallest size class (about 200 µm BW) were first observed in August 2016 in Kagoshima Bay and in early August 2015 and July 2016 on Amami-Oshima Island, which indicates that the recruitment period occurred almost one month later at Kagoshima Bay than on Amami-Oshima Island. This difference was likely caused by differences in reproductive seasons between two areas: on Amami-Oshima Island, the reproductive season is approximately one month earlier than in Kagoshima Bay.

Mature females with eggs of *S. erythraeensis* began to appear when the BW reaches approximately 700 µm in early spring, which was in March in Kagoshima Bay and in April on Amami-Oshima Island (Fig. 6), whereas males with spermatocytes were found slightly later than ovigerous females from June to August in Kagoshima Bay and from the end of May to July on Amami-Oshima Island (Fig. 8). It is possible that we did not observe the earliest development stages of spermatocytes as we only used an optical microscope for our observation of the coelom; thus, maturation may have started earlier in males. A histological study would be needed to examine the maturation process of females and males of *S. erythraeensis* in detail. The maturity rate of females was highest in the period from May to July 2016 in Kagoshima Bay, and from May to early July 2015 on Amami-Oshima Island (Fig. 7), and the mean oocyte size of each female increased to more than 80 µm by early summer. Males with spermatocytes and females with large eggs appeared simultaneously in early summer, which is therefore likely the time of reproduction in *S. erythraeensis*.

Seasonal changes in the appearance of mature males, maturity rates of females (Fig. 7), and oocyte sizes (Fig. 8) indicated that the reproductive season occurs approximately one month earlier on Amami-Oshima Island than in Kagoshima Bay. In some nereidid species, the reproductive season was reported to vary between regions. For example, reproductive swarming of *Hediste diadroma* Sato
Life history of *Simplisetia erythraeensis* & Nakashima 2003 was observed in winter or early spring at Kyushu and Honshu, but occurred in June at Hokkaido (Sato & Tsuchiya 1987, Sato & Nakashima 2003, Kikuchi & Yasuda 2006). Previous studies have shown that growth and reproduction of Annelida are influenced by diet and even more so by temperature (Levin & Creed 1986, Olive 1995, Olive et al. 1997). Sediment temperature at Shigetomi in Kagoshima Bay was lower than that at Ichi on Amami-Oshima Island throughout the year, apart from July and August when they were similar. This difference may have affected the timing of the respective reproductive seasons of *S. erythraeensis*. This species is distributed over a wide range along the Japanese coast and occurs as far north as Usu Bay, Hokkaido (approximately 42°13’N) (Imajima 1972). To examine the relationship between ambient temperature and the reproductive season of *S. erythraeensis*, the patterns of reproduction of population in the northern areas should be further investigated.

This study showed that individuals of *S. erythraeensis* begin to sexually mature at approximately 7 months after recruitment, reproduce at approximately 11 months after that, and die after reproduction. In South Africa, individuals of this species recruited from summer to autumn (December to April) probably reproduced during summer and died after reproduction (Kalejta 1992). Three types of life cycles are described in polychaetes annual, perennial, and multiannual (Fauchald 1983). The species *S. erythraeensis* is considered to belong to an annual category, i.e., an individual survives for only one year and dies after reproduction. However, further studies over a wide distribution range including the most northern habitats would be needed to comprehensively understand this species’ life cycle pattern.

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