A THESIS FOR THE DEGREE OF
MASTER OF SCIENCE

Annual gametogenesis pattern two scleractinian corals, *Alveopora japonica* and *Oulastrea crispata* from high-latitude Jeju Island, South Korea

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ABSTRAT

Populations of high-latitude screlactinian corals *Alveopora japonica* and *Oulastrea crispata* have been increasing recently in Jeju Island; however, no studies have investigated their reproductive physiology. Accordingly, with the use of histology, the present study, for the first time, examined the annual gametogenesis of those species in Korea. *A. japonica* and *O. crispata* were collected monthly from the north coast (Biyang-do, 33.1422.2N, 126.3525.1E) and the south coast (Bomok, 33.3313.5N, 126.41149E) of Jeju Island during 2015. Surface sea water temperature (SST) at the north sites ranged from 13.72 – 24.01℃, and 14.70 – 25.02 ℃ at the south annually. Histology revealed that the gonial cells (i.e., oogonia and spermatogonia) of *A. japonica* developed in separate mesenterial filaments of the polyps, however, the cells of *O. crispata* develop in same mesenterial filaments. In the north, *A. japonica*'s primordial oocytes first appeared in January, and the ripe eggs could be observed in August, 8 months after initiation of gametogenesis. Spermary development of *A. japonica* was relatively longer and showed irregular pattern, and the primordial spermaries first appeared in January with 9 months maturation in the north. On the south coast of Jeju Island, *A. japonica* oocytes were first observed in January, and could be seen in late August. As observed at the north site, speramary development pattern was comparatively regular, and the primordial spermaries first observed in January and had been matured for 9 months. Histology also demonstrated that *O. crispata* primordial oocytes first appeared in January, and the ripe eggs appeared in late September. In this study, we hypothesized *A. japonica* distributed at north and south had different spawning periods due to the different annual SST, however, histology suggests that *A. japonica* had same spawning period in late summer and early fall at both sites, also, hermaphroditic brooding species by observing the planulae. *O. crispata* were initiated in spring, and subsequent spawning occurred in late summer or early fall and hermaphroditic species but planulae could not observe.

국문요약

최근, 제주도 해역에서 고위도 돌산호종 거품돌산호(*Alveopora japonica*, Eguchi, 1968) 와 *Oulastrea crispata* 의 개체 수는 꾸준히 증가하고 있는 것으로 보고되었으나 돌산호종들에 대한 번식 생리학적 연구는 미비한 실정이다. 이에 따라 *A. japonica* 와 *O. crispata* 의 연중 번식주기 연구를 조직학적 관찰을 통해 진행하였다. 본 연구에서는 거품돌산호(*A. japonica*) 와 *O. crispata* 는 제주 북쪽 (비양도, 33.1422.2N, 126.3525.1E)과 남쪽 (보목, 33.3313.5N, 126.41149E) 해역에서 매월 시료를 채집하였고, 조사해역의 평균 표층수온 (SST)은 북쪽에서는 약 13.72 ~ 24.01 ℃, 그리고 남쪽 해역은 14.70 ~ 25.02 ℃로 조사되었다. 조직학적 관찰 결과, *A. japonica* 의 난모세포와 정모세포는 하나의 산호 폴립 내 각각 다른 격막사 (mesenterial filament) 내에서 성숙하고, *O. crispata* 의 생식세포는 동일한 격막사에서 따로 성숙하는 것으로 확인되었다. 북쪽 해역의 *A. japonica* 의 연중 번식주기 관찰 결과, 초기 난모 세포는 산호 시료 채집을 시작한 2015 년 1 월부터 관찰이 되었으며 성숙한 난자는 초여름인 8 월부터 관찰이 되기 시작하였다. 북쪽 해역의 거품돌산호(*A. japonica*)는 약 8 개월간의 성숙 시간을 거친 뒤 산란하는 것으로 관찰 되었다. 정모세포의 경우, 1 월부터 관찰되었으며, 약 9 개월간의 성숙기를 거쳐 방출하는 것으로 확인되었다. 남쪽 해역의 거품돌산호(*A. japonica*)의 초기 난모세포도 1 월부터 관찰되었으며 8 월말까지 성숙하는 것으로 확인되었다. 남쪽해역에서만 채집된 *O. crispata 의* 경우, 초기 난모세포는 거품돌산호(*A. japonica*)와 마찬가지로 1 월부터 관찰되었으며, 9 월부터 산란하는 것을 확인하였다. 본 연구에서는 북쪽과 남쪽에 서식하는 거품돌산호(*A. japonica*)의 연중 번식주기 연구를 조직학적 관찰을 통해 진행하였으며, 주 산란은 늦여름부터 초가을까지 진행하는 것으로 확인되었다. 또한, 거품돌산호(*A. japonica*)와 *O. crispata* 는 자웅동체이며, 거품돌산호(*A. japonica*)에서 방출된 유생까지 관찰함으로써 Brooding species 인 것까지 확인하였다.

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1. Introduction

Understanding the reproductive traits of scleractinian corals is important for managing and maintaining the coral population (Harrison 2011). Reproductive study includes the formation, growth and development of gametes (oogenesis and

spermatogenesis), spawning and fertilization, and larval development (Olive 1985). Out of reported 1500 scleractinian coral species, approximately 30% of species' reproductive traits such as sexual reproduction and larval development have been identified (Harrison 2011).

Corals have simple life cycle with a dominant benthic stage and a shorter planula larval period. Coral reproduction involves asexual reproduction that produces genetically identical clones and sexual reproduction that can produce genetic recombination and new coral genotypes. Sexual reproduction is categorized into four patterns: hermaphroditic broadcast spawners, hermaphroditic brooders, gonochoric broadcast spawners, or gonochoric brooders (Harrison 2011). Reproductive patterns vary across the coral distribution range (Mangubhai and Harrison 2008; Madsen et al. 2014; Eyal-Shaham et al. 2016). Corals distributed in the tropical and subtropical areas usually have two gamatogenic cycles per year, while corals in high-latitude area have only one gametogenic cycle annually (Mangubhai and Harrison 2008).

Alveopora japonica (Eguchi 1968) is a zooxanthellate scleractinian coral distributed in Korea (Jeju Island) and Japan (Eguchi and Fukuda 1972; Eguchi 1973; Song 1991; Nomura et al. 1994; Nishihira and Veron 1995; Veron 2000; Choi and Park 2001; Song et al. 2003; Yamano et al. 2004; Sugihara et al. 2009; Sugihara et al. 2014; Song 2004; Denis et al. 2015; Vieira et al. 2016). Populations of *A. japonica* usually inhabit shallow areas, and growth may be inhibited by environmental stress, such as wave and wind (Harii et al. 2001; Sugihara et al. 2014; Vieira et al. 2016). Colonies of *A. japonica* are hemispherical in shape, discrete, and usually measure 2-3 cm in diameter, but often can be larger, more than 10cm in diameter (Harii et al. 2001; Sugihara et al. 2014; Vieira et al. 2016). In addition, *A. japonica* is a hermaphroditic brooder; each polyp possesses both male and female gonadal functions (oocyte and speramary), and release planula larvae through internal fertilization during September and October (Igarashi et al. 1992; Harii et al. 2001; Thamrin et al. 2001; Sugihara et al. 2014; Eyal-Shaham et al. 2016; Vieira et al. 2016).

On the other hand, *Oulatrea crispata* (Lamark 1816) is widely distributed from the Great Barrier Reef in the southern hemisphere to Japan in the northern hemisphere (Nemenzo 1986; Veron 1986; Yajima et al. 1986; Lam 2000). *O. crispata* has been reported to possess outstanding low-temperature tolerance; colonies have been found where the sea surface water temperature ranged from 7-10 °C during winter in the Philippines and in Japanese waters (Yajima et al. 1986; Lam 2000; Veron 2000; Chen et al. 2011). *O. crispata* known as "Zebra coral" because of its external features with a distinctive black and dark brown skeleton with white upper margins extending to the radiating septa (Kawaguti and Sakumoto 1952; Lam 2000; Chen et al. 2011). Colonies form encrusted, sub-massive forms, and usually grow a few centimetres across but often reach about 10cm (Kawaguti and Sakumoto 1952; Yajima et al. 1986; Lam 2000; Chen et al. 2011). *O. crispata* has been reported to be a hermaphroditic brooding species, releasing planulae within an extended reproductive period from July to October (Nakano and Yamazato 1992; Lam 2000).

During last decade, poleward migration of corals has been reported at high latitude Japan and Korea (Veron 2000; Harii et al. 2001; Park and Kang 2010; Yamano et al. 2011; Denis et al. 2013, 2015) but, information on reproduction has been carried out by very few studies. Most of the work has been carried out in tropical and subtropical area (Lam 2000; Harii et al. 2001; Madsen et al. 2014) including Japan, China, Hong Kong and Taiwan (Lam 2000; Thamrin et al. 2001; Harii et al. 2001).

Jeju Island is affected by the Tushima Current, a branch of the Kuroshio Current, and features high-latitude environmental conditions with the surface sea water temperature (SST) in the subtidal area ranging between 13 to 18 °C during the winter (Lie et al. 2000; Ichikawa and Beardsley 2002; Veron 2011; Sugihara et al. 2014; Palmas et al. 2015). During the past 100 years, the SST around Korea has increased by 1.2 – 1.3 °C due to global warming (Lie et al. 2000; Abu Affan and Lee 2004; Takatsuki et al. 2007; Sugihara et al. 2014). Coinciding with increased SST, the environmental conditions of high-latitude locations are expected to experience changes (Yamano et al. 2011; Denis et al. 2013, 2015;

Chang et al. 2014; Hong et al. 2015; Palmas et al. 2015; Vieira et al. 2016). Among the various environmental factors, temperature has the strongest impact on marine organisms (Yamano et al. 2011; Denis et al. 2013, 2015; Vieira et al. 2016). In the past, coral distribution was restricted to tropical and subtropical areas; however, corals also inhabit temperate area without building coral reefs (Yamano et al. 2011; Vieira et al. 2016). For the past 20 years, *A. japonica* has been commonly found in Jeju and formed large populations on the north and south coasts of the island (Song 1991, 2004; Park and Choi 2001; Denis 2013, 2015; B. I. Kim, local SCUBA diving shop, pers. comm; Vieira et al. 2016); however, *O. crispata* has been reported only in the south (Park and Choi 2001). Despite the emergence of scleractinian corals, soft corals and azooxanthellate corals have been extensively investigated while studies of Jeju hard corals and zooxanthellate corals are deficient (Song 1991; Song et al. 2003; Park and Choi 2001; Hwang and Song 2009; Sugihara et al. 2014).

The aims of the present study were to investigate the hypothesis that the high-latitude scleractinian corals *A. japonica* and *O. crispata* maintain their local population through annual gametogenesis, and have different reproductive periods depending on environmental conditions, specifically surface seawater temperature in the north and south. We examined annual (cyclic) gametogenesis, reproductive period, and gonad development through histology to understand reproductive characteristics in high-latitude coral species.

2. Materials and Methods

2.1. Study area and coral sampling

Jeju is situated off the south of the Korean Peninsula (Won 1976; Sugihara et al. 2014) and the eastern coast faces Tsushima and Nagasaki in Japan across the South Sea of the Korea Peninsula and the East China Sea (Won 1976; Sugihara et al. 2014). In addition, the western coast of Jeju faces Shanghai in China across the East China Sea. Due to its location, the annual SST shows about 0.5 – 1.0 ℃ difference at the north and south in summer season. Coral samples were collected from one site each along the northern (Biyangdo – 33.1422.2N, 126.3525.5E) and southern (Bomok – 33.3313.5N, 126.4114.9E) coasts of Jeju Island (Fig. 1). *A. japonica* was collected from both locations; however, *O. crispata* only occurred in the southern coast. From January to December 2015, three to five colonies were collected monthly at each site from a depth of 10m by SCUBA diving, using chisel and hammer. Whole colonies of *A. japonica* were collected because the species usually forms solitary colonies protruding from the substrate. However, *O. crispata* specimens were collected partially from the entire colony because the species spreads along the substrate. In August and September, during the predicted reproductive periods, sampling was carried out twice a month. *A. japonica* and *O. crispata* fragments after collection were immediately fixed in 10 % seawater-formalin solution for a period of 48 hrs and finally transferred to glass bottles with 70% ethanol.

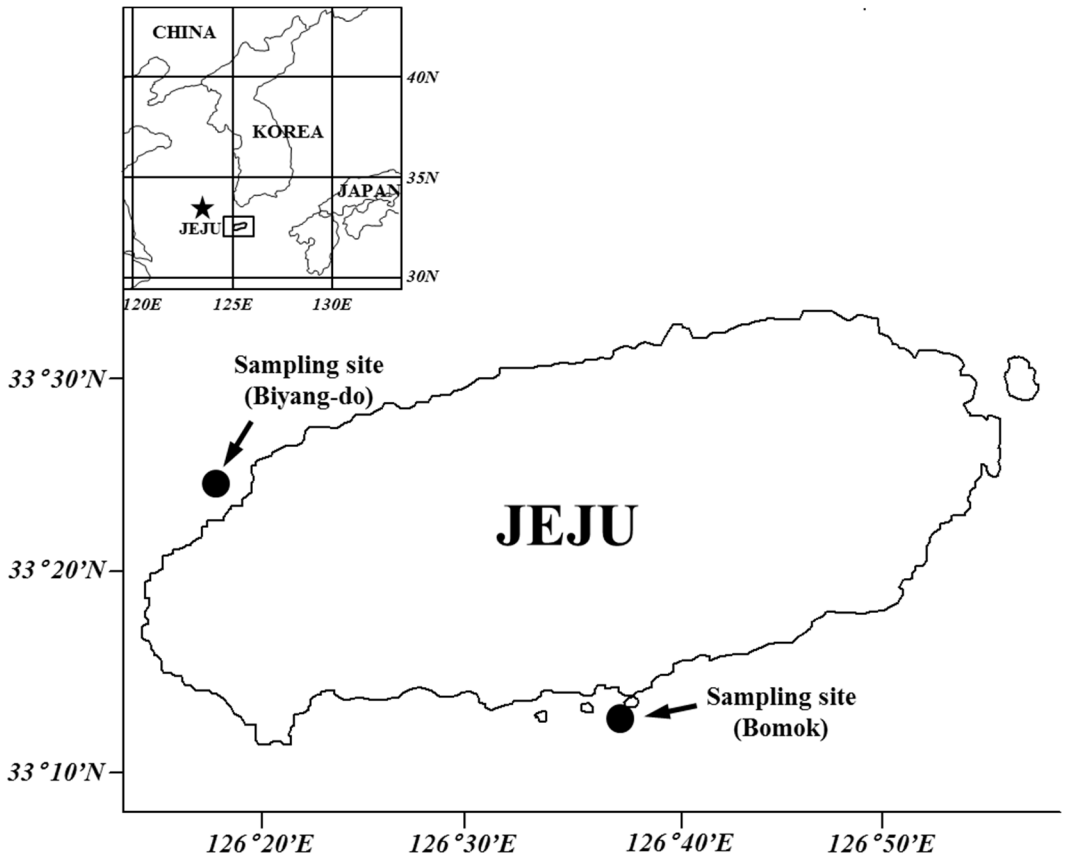

Figure 1. Map of Jeju Island (marked with star). Two sampling sites Biyang-do and Bomok in Jeju Island (marked with black dots)

2.2. Environmental parameters

SST was monitored at depth of 5m at sampling sites by installing HOBO temperature loggers (HOBO Pendant temperature /light data logger, Onset Computer Corp., USA) from March to December, 2015 (Fig. 2). *A. japonica* and *O. crispata* were collected beginning in January, 2015; however, surface seawater temperature data for the first two months were absent due to uninstallation of the logger. At the northern site, SST was the lowest in March, at 13.72 ± 0.45 °C and the highest in August as 24.01 ± 1.37 °C during the research period. At the southern site, the lowest SST was recorded in March, at 14.70 ± 0.23 °C and the highest in August as 25.02 ± 1.52 °C.

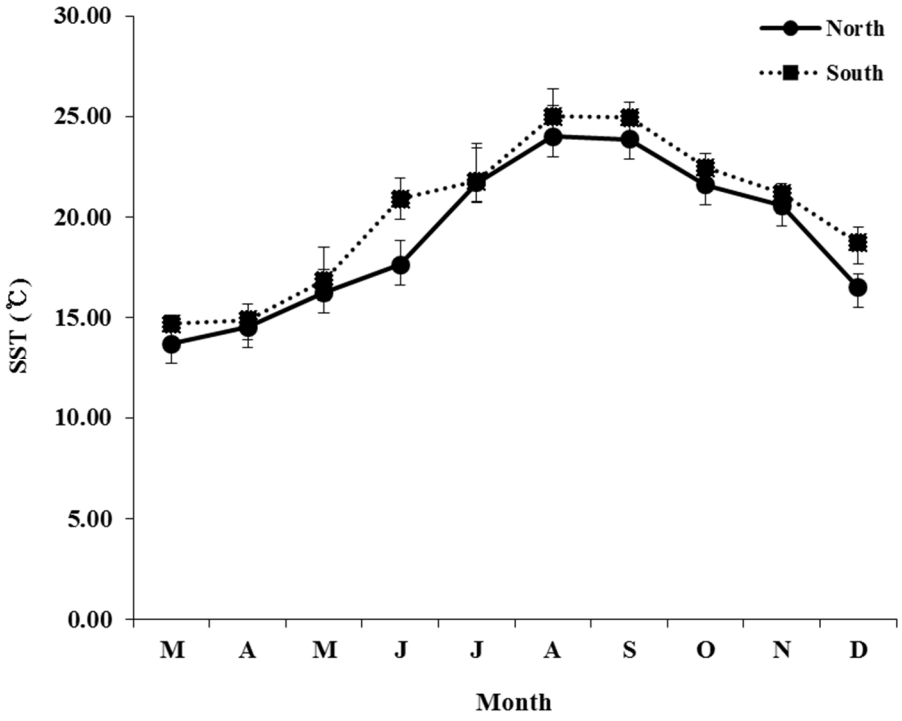

Figure 2. Seasonal variation of surface seawater temperature (SST) from the sampling site (northern and southern coasts)

2.3. Histology

Formalin fixed samples were transferred to 70 % ethanol for a week with repeated changes of alcohol, for decolorizing and eliminating impurities. Coral specimens were then divided into three sub-parts (each with > 10 polyps) and then decalcified. Decalcified coral using a solution consisting of 20 % citric acid and 50 % formic acid. Decalcification continued until bubbles failed to appear on the tissue of the coral with the continuous changing of the solution at 5 minute intervals. The soft coral tissues were cut into 1cm^2 and were fixed using Davision's fixation solution for 24 hrs. The samples were then dehydrated through a concentration gradient of ethanol within a tissue processor machine. After dehydration, tissues were embedded in paraffin and sectioned longitudinally at a thickness of 6 µm. Monthly samples produced a total of 225 slides (75 slides for the northern *A. japonica* and 115 slides for the southern *A. japonica* and *O. crispata* locality). The tissue section were stained using standard protocol for *Harris's Hematoxylin* and *eosin* method.

2.4. Analysis of reproductive stage

Longitudinally sectioned (LS) slides were analysed under a polarized light microscope (AXIO Scope, A1 produced by ZEISS, Germany) at magnifications of x5, x10, x20 and x40, and photographs were taken by microscope cam (AXIO Scope, A1 by ZEISS, Germany). Gametogenesis (oogenesis and spermatogenesis) was categorized into five developmental stages (Szmant-Froelich et al. 1985); 0) empty and indifferent, 1) primordial stage, 2) early development, 3) late development, and 4) ripe and ready for spawning. The longest diameter (D1) and the second measurement perpendicular to the longest diameter (D2) were measured with image analysis programs (Image J, National Institutes of Health, USA and Photoshop CS 5.1, Adobe, USA), and the geometric mean diameter (GMD) of each gonad was calculated (Guest et al. 2012):

$$\text{GMD} = \sqrt{(\text{D1} \times \text{D2})}$$

In addition, the surface area of each gonad was calculated by measuring the outline of the both sexual gonad (oocytes and spermary), except the epithelium using the image J program. The surface area of the gonads was also used as an indicator of reproductive development in addition to GMD.

2.5. Observation of planulae release

A total of 12 colonies, including 3 live colonies, were additionally collected and investigated for planulae release on August 19th and September 1st, which were predicted reproductive periods. Corals were reared in the laboratory in flow-out aquarium (30 × 20 ×13 cm) with running seawater. The outlets of the aquariums were covered with 100 μm meshes for collecting released planulae. Environmental conditions in the aquarium were set coinciding with the conditions of the sampling sites by filling the aquariums with filtered seawater, and maintaining a water temperature from 22 – 24 ℃. In addition, 12/12 h light/dark conditions were regulated for photosynthesis of the corals. The seawater of the aquariums was renewed every two days. When the planulae emerged from the coral colonies, they were collected from the meshes and transferred by pipette to petri dishes filled with seawater. For scanning electron microscope (SEM, SUPRA-55VP, Carl Zeiss, Germany) observation, the captured planulae were fixed in 2.5 % glutaraldehyde at 4°C for overnight. After fixation, the planulae were dehydrated in an ethanol series and processed in an iso-amyl acetate series. Then, the planulae were coated with platinum and observed with a microscope at an accelerating voltage of 15kV.

3. Results

3.1. Gonadal development in *A. japonica* and *O. crispata*

Histology revealed that *A. japonica* and *O. crispata* had annual gametogenesis and are hermaphroditic species (Fig. 3 & 4). Oocytes and spermaries of *A. japonica* developed in separate mesenterial filaments, however, sexual gonads of *O. crispata* were found in same mesenteries. Gametogenesis into developmental stages for *A. japonica* and *O. crispata* was classified into different stages (Szmant-Froelich et al. 1985) (Fig. 3 & 4). Stage 0, no ova in mesenteries and only empty spaces (Fig. 3 & 4. (A)). Stage 1, primordial oocytes had enlarged interstitial cells with visible nuclei in mesoglea of the mesenteries (Fig. 3 & 4. (B)). Stage 2, cytoplasm accumulated around the nuclei (Fig. 3 & 4. (C)), and stage 3, variable sizes of oocytes, and vitellogenesis developed mainly in this period (Fig. 3 & 4. (D)). In stage 4, the maximum size of oocytes were observed clearly in mesenteries with indented nucleus, distinguished vitelline membrane and had full size (Fig. 3 & 4. (E)). The spermaries showed different features in development stages. In the empty stage (stage 0), most mesenteries were empty and spermatocytes were not observed (Fig. 3 & 4. (F)). In the primordial stage (stage 1), small clusters of interstitial cells (spermatocytes) began to enter and be enlarged in mesoglea (Fig. 3 & 4. (G)). In early development stage (stage 2), clusters of spermatocytes had been elongated and were observed with distinct spermary boundaries (Fig. 3 & 4. (H)). In late development stage (stage 3), spermatocytes with small nuclei and large number of cells within spermaries were observed (Fig. 3 & 4. (I)). In the ripe stage (stage 4), spermatocytes with little amount of cytoplasm were observed in lumen (Fig. 3 & 4. (J)).

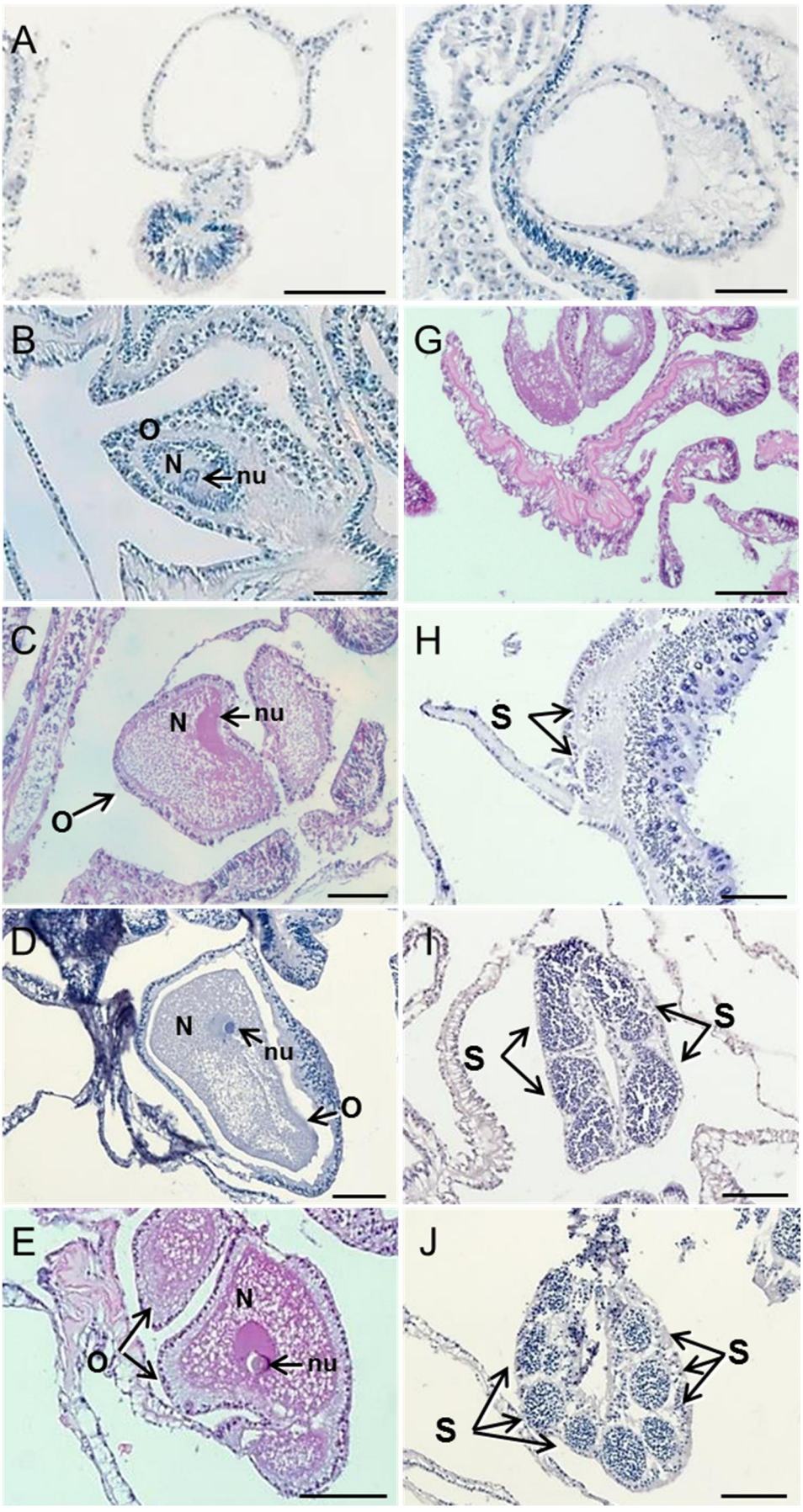

Figure 3. Transverse sections through the gonads of A. japonica. (A) Empty space in mesenteries after egg releasing (Scale bar= 100μm). (B) Primordial oocyte (Scale bar= 50μm). (C) Oocytes in early development stage (Scale bar= 100μm). (D) Oocytes in late development stage (Scale bar= 50μm). (E) Mature oocytes (Scale bar= 100μm). (F) Empty testes after spawning (Scale bar= 50μm). (G) Primordial spermaries (Scale bar= 100μm). (H) Spermaries in early development stage (Scale bar= 50μm). (I) Spermaries in late development stage (Scale bar= 50μm). (J) Mature spermaries (Scale bar= 50μm). O: oocyte; N: nucleus; nu: nucleolus; S: spermary

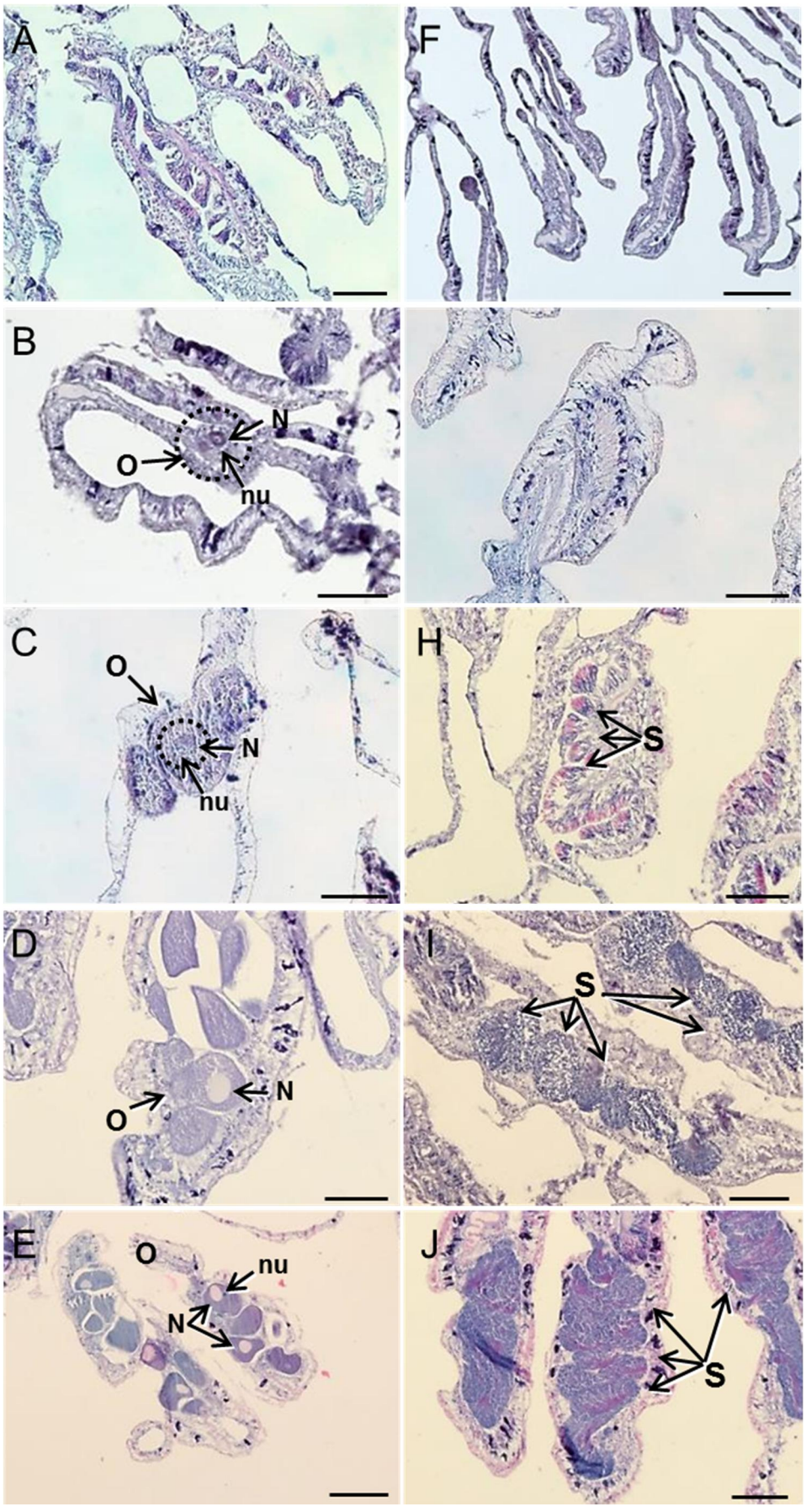

Figure 4. Transverse sections through the gonads of O. crispata. (A) Empty space in mesenteries after egg releasing (Scale bar= 50μm). (B) Primordial oocyte (Scale bar= 50μm). (C) Oocytes in early development stage (Scale bar= 50μm). (D) Oocytes in late development stage (Scale bar= 50μm). (E) Mature oocytes (Scale bar= 100μm). (F) Empty testes after spawning (Scale bar= 50μm). (G) Primordial spermaries (Scale bar= 50μm). (H) Spermaries in early development stage (Scale bar= 50μm). (I) Spermaries in late development stage (Scale bar= 50μm). (J) Mature spermaries (Scale bar= 50μm). O: oocyte; N: nucleus; nu: nucleolus; S: spermary

3.2. North *A. japonica*

3.2.1. Oogenesis

Histology indicated that oogenesis of *A. japonica* commenced in January 2015 respectively when the SST was less than 13.72 °C and 14.83 °C (SST of February) in the North and South respectively (Fig. 2). Oocytes developed in the following months, reaching a maximum gonad size and maturity in July and August. The detectable primordial oocytes in the early development stage had a mean GMD (geometric diameter) of 64.83 ± 17.69 μm (n = 32) and a surface area of 3564.40 ± 1704.93 μm^2 (n = 32) and were first observed in January 2015 (Fig. 5). The proportion of *A. japonica* in the early development stage increased, and reached its maximum in February 2015 at 45.47 % (Fig. 6, Left). The oocytes in the late development stage were first observed in January, with primordial oocytes, and the proportion at this stage reached its maximum in February 2015 at 45.47 % (Fig. 6, Left). The oocytes in the late development stage were first observed in January, with primordial oocytes, and the proportion at this stage reached its maximum in April (13.64%), (Fig. 6, Left). Mature *A. japonica* oocytes first observed in March had a mean GMD of 56.74 ± 20.70 μm (n = 5) and a surface area of 3077.98 ± 2407.74 μm^2 (n = 5) (Fig. 5). Oocytes then increased in the following 5 months in August having a mean GMD of 172 ± 43.57 μm (n = 28), and a surface area of 24364.89 ± 10940.2 μm^2 (n = 28) (Fig. 5). The proportion of the mature stage began to increase from March (1.59 %) and was dominant from July (80.77 %) to August (94.12 %) (Fig. 6, Left). Oocytes in mature stage had been observed during half of the research period (6 months) from March to August. Major spawning activity was observed between August and September respectively when SST ranged from 24.01 ± 1.37 ~ 23.89 ± 0.74 °C (Fig. 2). The proportion at the mature stage decreased remarkably from 94.12 % to 0 % in September (Fig. 6, Left). In November and December, only empty oocytes were observed, indicating releasing of eggs.

The number of polyps and oocytes was counted in local union 1cm^2 for calculating those densities. The polyp density was 10 ± 0.83 (n'=4, n'=observed slides #) in January and reached maximum density at 13 ± 1.89 (n'=3) in September (Table 2). However, oocyte density was 5 ± 1.7 (n'=3) in January and reached maximum 7 ± 2.77 (n'=4) in July (Table 2). Between August and September, when the major spawning activity occurred, the polyp density increased from 9 ± 2.35 (n'=4) to 13 ± 1.89 (n'=3), while the oocytes decreased from 3 ± 1.7 (n'=6) to 2 ± 2.14 (n'=6) (Table 2).

3.2.2. Spermatogenesis

The spermatogenesis of *A. japonica* began in January 2015, with the same timing of oogenesis. Spermaries matured in the following periods, reaching a maximum gonad size and a surface area in September (Fig. 5). In January, spermaries in all development stages were observed, and had a mean GMD of 17.53 ± 3.22 μm (n=8) and a surface area of 286 ± 78.60 μm^2 (n = 8) (Fig. 5). Only spermaries in empty stage were observed in February. The proportion of *A. japonica* in early development stage was increased and reached a maximum at 34.57 % in May (Fig. 8, Right). The spermaries in the late development stage were first observed in January, and the proportion at this stage reached its maximum in September (44.12 %) (Fig. 6, Right). Mature spermaries were first observed in January, and had a mean GMD of 17.53 ± 3.22 μm (n=8) and a surface area of 286 ± 89.60 μm (n=8) (Fig. 5). Spermaries then developed in the following 8 months in September with a mean GMD of 81.13 ± 22.19 μm (n=8) and a surface area of 5873.93 ± 3170.84 μm^2 (n=8) (Fig. 5). The proportion at mature stage increased from May (14.81%) and became dominant in July (86.79%) and August (62.63%) (Fig. 6, Right). Major spawning activity began in July when the SST was 21.72 ± 1.87 °C (Fig. 2), and was observed from July (proportion of mature stage: 86.79 %) to October (proportion of mature stage: 0 %) (Fig. 6, Right). From October to December, only empty spermaries were observed indicating spawning. The density of spermary was 3 ± 1.25 (n=3) in January and reached a maximum of 17 ± 9.62 (n=5) in July (Table 2). Between July and October, when the major spawning occurred, the number of spermaries dramatically decreased from 17 ± 9.62 (n=5) to 1 ± 1.22 (n=4) (Table 2).

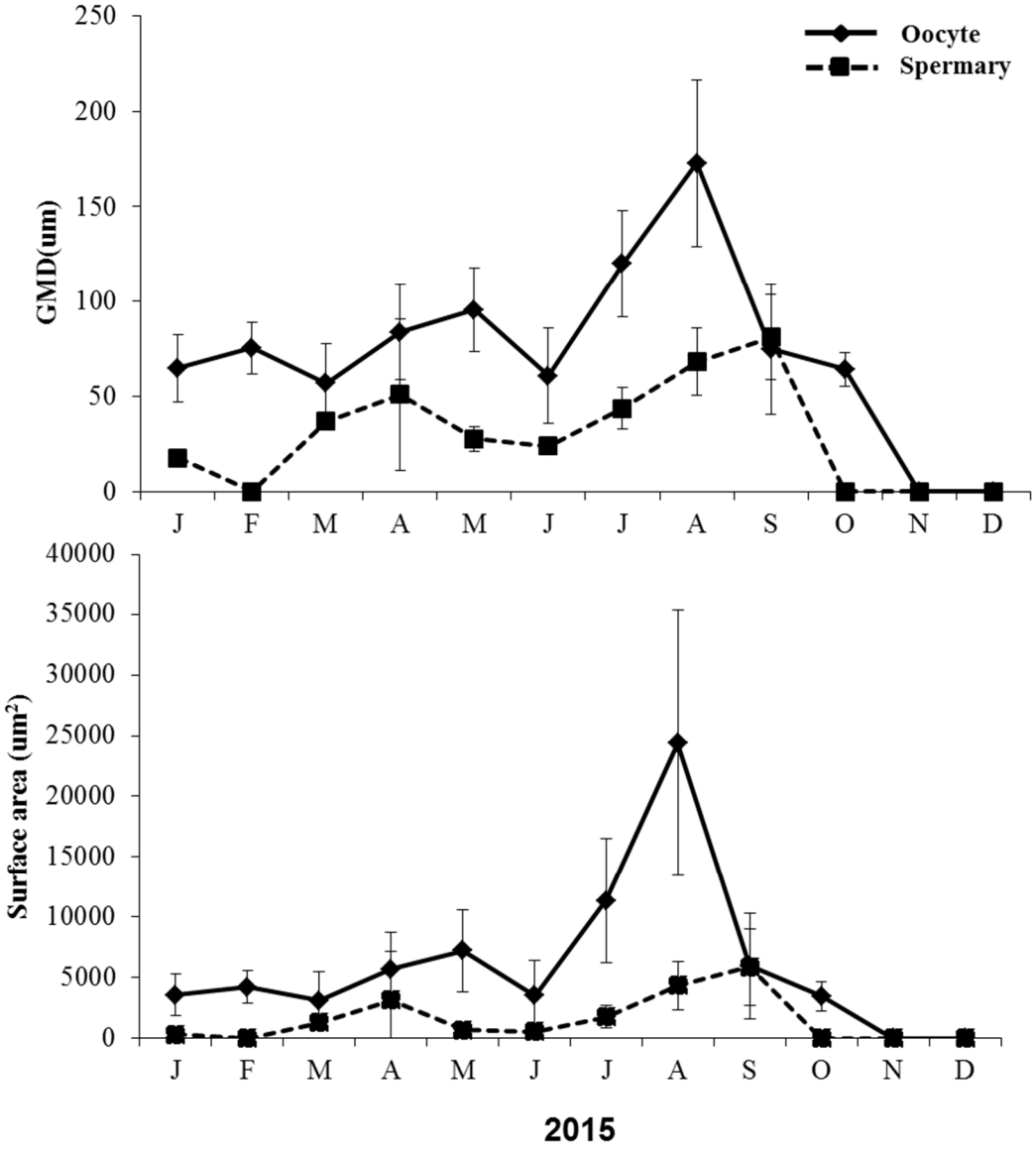

Figure 5. Seasonal changes of GMD (Geometric diameter) and surface area for both sexual gonads of A. japonica from the northern coast.

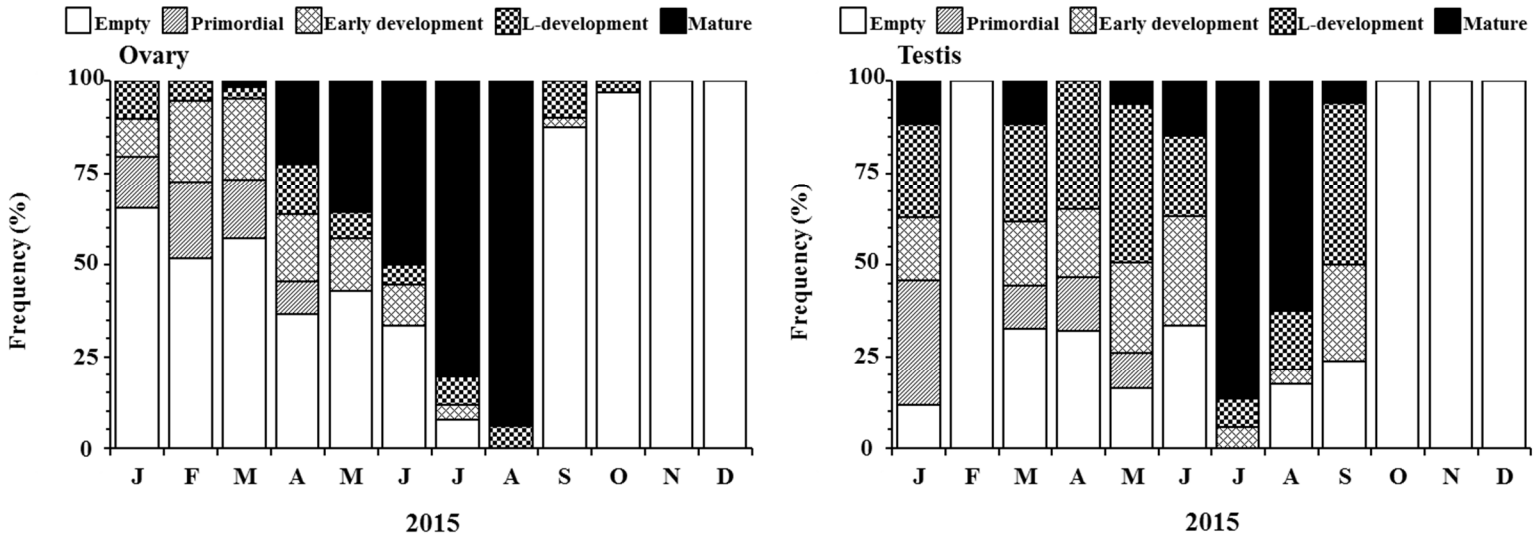

Figure 6. Percentage of A. japonica from the northern coast in various reproductive stages during study period

3.3. South *A. japonica*

3.3.1. Oogenesis

Histology indicated that oogenesis of *A. japonica* commenced in January, when the SST was less than 14.83 °C (SST of February) (Fig. 2). Oocytes developed in the following 8 months, having a maximum gonad size and maturity in August. The detectable primordial oocytes in the early development stage had a mean GMD of 72.58 ± 16.22 μm (n=17) and a surface area of 4478.78 ± 1883.01 μm^2 (n=17), and were first observed in January, at the same time as the northern *A. japonica* (Fig. 7). The proportion of *A. japonica* in the early development stage was increased and became dominant in March (50%) (Fig. 8, Left). The oocytes in the late development stage were first observed in February, with primordial oocytes, and the proportion at the stage reached its maximum in June (26.83%) (Fig. 8, Left). Mature oocytes were first observed in April and had a mean GMD of 82.44 ± 22.91 μm (n=16) and an area of 5736.77 ± 2770.49 μm (n=16) (Fig. 7). Oocytes then increased in 4 months, reaching a mean GMD of 166.22 ± 35.22 μm (n=16) and a surface area of 22067.64 ± 9021.94 μm^2 (n=16) in August (Fig. 7). The proportion at the mature stage began increasing from April and became dominant in July (53.85%) and August (82.61%) (Fig. 8, Left). Major spawning activity was observed between August and September respectively when SST ranged from 24.95 ± 0.79 ~ 25.02 ± 1.52 °C (Fig. 2). The proportion of mature oocytes dramatically decreased from 82.61 to 0 % in September (Fig. 8, Left). In October and November, only oocytes in the empty stage were observed, indicating the release of eggs. Polyp density was 12 ± 2.86 (n=4) in January and reached its maximum of 19 ± 2.49 (n=3) in August (Table 2). However, oocyte density was 4 ± 3.56 (n=4) and reached its maximum 5 ± 5.62 (n=5) in May (Table 2). Between August and September, when the major spawning occurred, the polyp density decreased from 19 ± 2.49 (n=3) to 8 ± 1 (n=2) (Table 2). Oocyte density decreased from 3 ± 0.8 (n=5) to 2 ± 1.25 (n=3) (Table 2).

3.3.2. Spermatogenesis

Histology revealed that spermatogenesis began in January 2015. Spermaries matured in the following periods, reaching a maximum gonad size and a surface area in August (Fig. 8). In January, spermaries in all stages, except the mature stage, were observed, and had a mean GMD of 44.68 μm (n=1) and a surface area of 1643.70 μm (n=1), (Fig. 7). The proportion of *A. japonica* in the early development stage reached its maximum at 62.96 % in March (Fig. 8, Right). The spermaries in the late development stage were first observed in January, and the proportion at the stage reached its maximum in April (41.07%) (Fig. 8, Right). Mature spermaries first observed in May had a mean GMD of 19.46 ± 3.85 (n=4) and a surface area of 374.01 ± 153 μm^2 (n=4) (Fig. 7). Spermaries developing in the following 4 months to September had a maximum mean GMD of 108.89 ± 14.81 μm (n=2) and a surface area of 10877.84 ± 3742.35 μm^2 (n=2) (Fig. 7). The proportion at the mature stage increased from May (3.45 %) and became dominant in July (57.14%) and August (59.52%) (Fig. 8, Right). Major spawning activity began in August, when SST was 25.02 ± 1.52 °C (Fig. 2), and was observed from August (proportion of mature stage: 59.52%) to October (proportion of mature stage: 0%) (Fig. 8, Right). From October to December, only empty stage spermaries were observed that indicated spawning activity was finished. Spermary density was 2 ± 2.16 (n=4) in January and reached its maximum 12 ± 11.15 (n=3) in July (Table 2). Between August and October, when major spawning occurred, spermary density decreased from 4 ± 1.92 (n=4) to 0 (n=3) (Table 2).

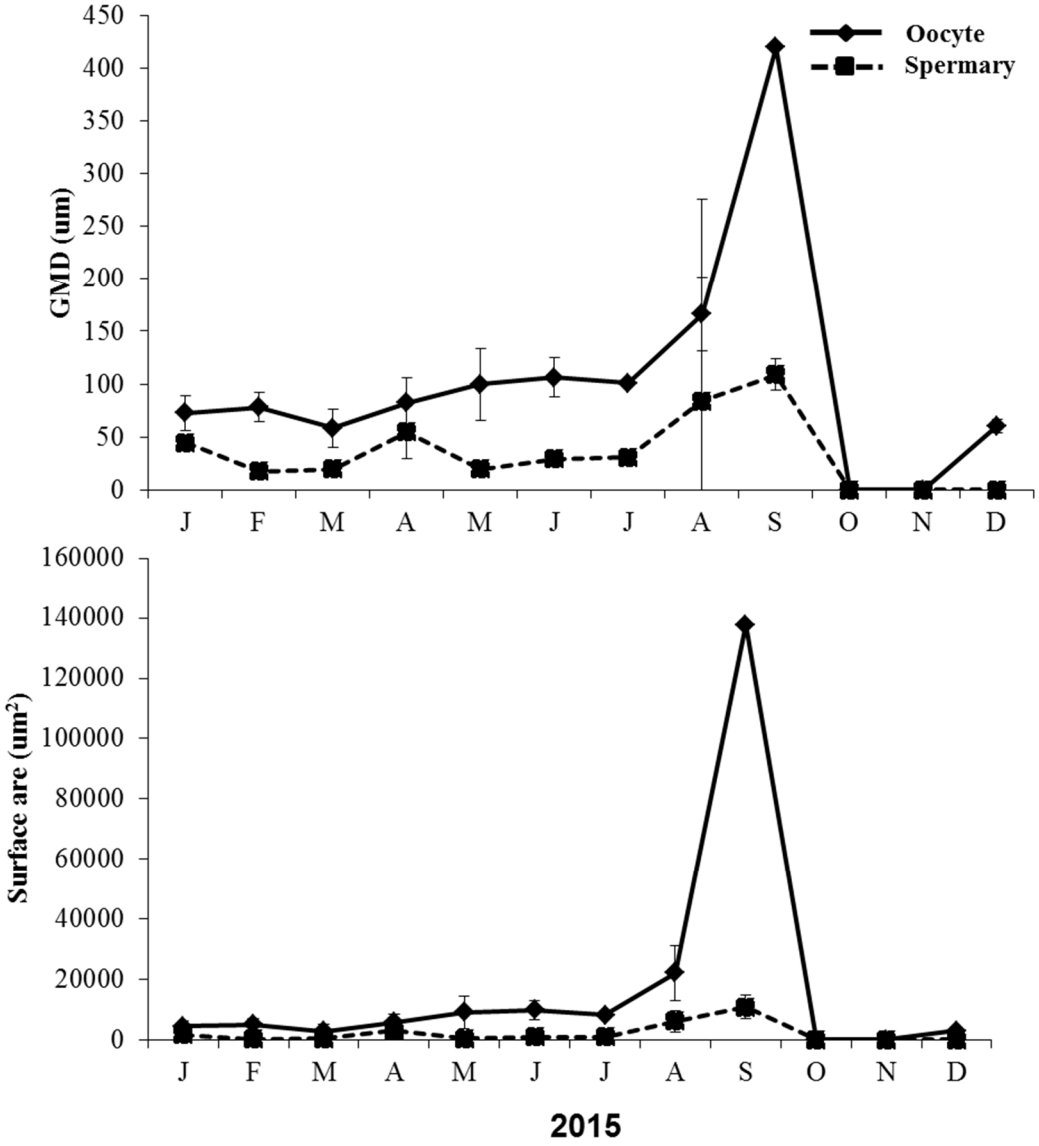

Figure 7. Seasonal changes of GMD (Geometric diameter) and surface area for both sexual gonads of *A. japonica* from the southern coast

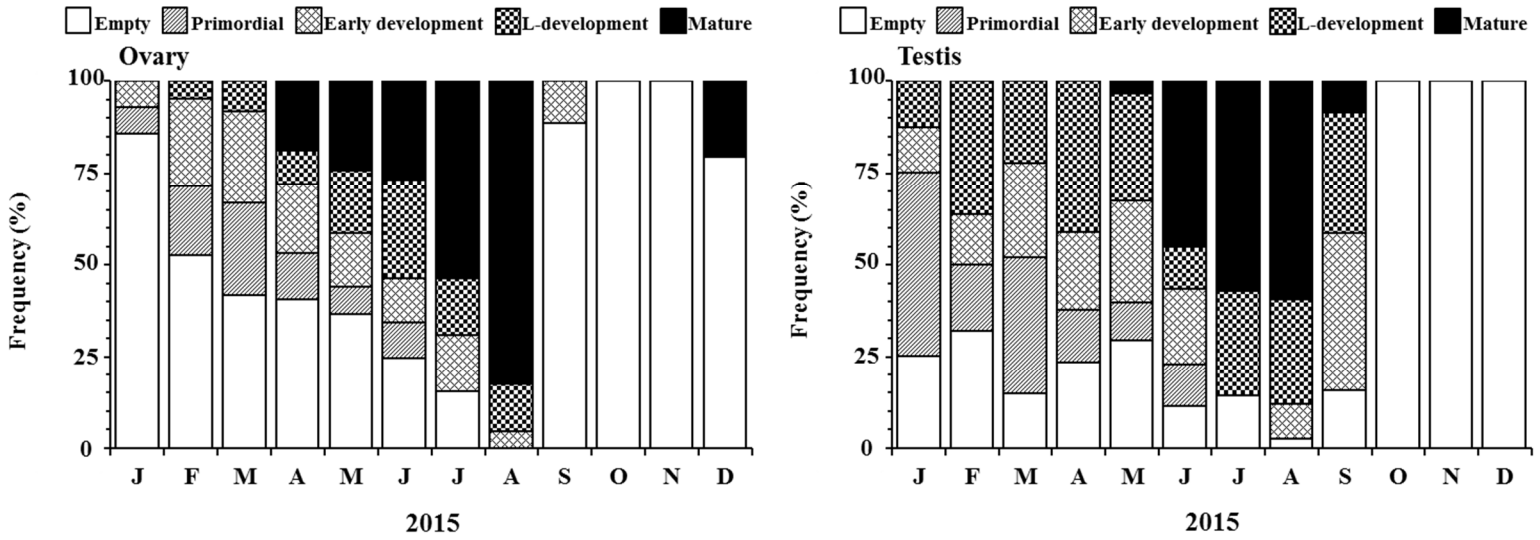

Figure 8. Percentage of *A. japonica* from the southern coast in various reproductive stages during study period

3.4. South *O. crispata*

3.4.1. Oogenesis

Histology revealed that oogenesis of *O. crispata* commenced in January when the SST was less than 13.72 °C (Fig. 2). Oocytes were developed in the following months, reaching a maximum gonad sized and maturity in August. The detectable primordial oocytes in the early development stage, having a mean GMD of 19.78 ± 9.02 μm (n=5) and a surface area of 396.94 ± 364.58 μm^2 (n=5), were first observed in January 2015 (Fig. 9). The proportion of *O. crispata* in the early development stage increased and reached its maximum in April at 34.62 % (Fig. 10, Left). Oocytes in the late development stage were first observed in March, and the proportion at this stage reached its maximum in July at 80.95 % (Fig. 10, Left). Mature *O. crispata* oocytes, first observed in May, had a mean GMD of 47.19 ± 14.39 μm (n=26) and a surface area of 2183.36 ± 1279.3 μm (n=26) (Fig. 9). The proportion at mature stage began increasing from May (4.17%) and became dominant in September (78.57%) (Fig. 10, Left). Matured oocytes were observed for 5 months from May to September. Major spawning activity was observed between September and October, when SST ranged from 22.47 ± 0.44 ~ 24.95 ± 0.79 °C (Fig. 2). The proportion at the mature stage decreased remarkably from 78.57% to 0% (Fig. 10, Left). In November and December, only empty female gonads existed, indicating the finished release of eggs. Polyp density was 7 ± 1.48 in January and reached its maximum at 9 ± 2.36 in October (Table 2). However, the density of oocytes was 3 ± 2.97 in January and reached its maximum at 33 ± 28.06 in August (Table 2). Between September and October, when the major releasing activity occurred, polyp density increased from 7 ± 0.47, while oocyte density decreased form 17 ± 8.64 to 0 (Table 2).

3.4.2. Spermatogenesis

According to histology, spermatogenesis began in March, 2 months later than oogenesis. Spermaries matured in the following periods, reaching a maximum gonad size and a surface area in August, when the SST was 25.02 ± 0.79 °C (Fig. 2). In January and February, only empty spermaries were observed. Spermaries in the early development stage were first observed in March, and had GMD of 37.37 ± 9.48 μm (n=3) and a surface area of 1216.93 ± 478.44 μm (n=3) (Fig. 9). The proportion of *O. crispata* in early development stage varied and reached its maximum in April at 17.39 % (Fig. 10, Right). The spermaries in the late development stage were first observed in April and the proportion became dominant in July at 73.91 % (Fig. 10, Right). Mature spermaries first observed in July had a mean GMD of 20.43 ± 4.19 μm (n=4) and a surface area of 400.03 ± 192.63μm (n=4) (Fig. 9). Mature spermaries developed in the following month, in August, had a maximum GMD of 42.99 ± 12.51 μm (n=8) and a surface area of 2056 ± 1263.3 μm^2 (n=8) (Fig. 9). The proportion at the mature stage increased in the following 3 months and became dominant in October at 80 % (Fig. 10, Right). Major spawning activity occurred between October (proportion at mature stage: 80%) and November (proportion at mature stage: 0%) (Fig. 10, Right) respectively, when SST ranged 21.19 ± 1.09 ~ 22.95 ± 0.79 °C (Fig. 2). In November and December, mature spermaries were not observed while early and late development stages were observed, indicating that spawning was finished. Spermary density was 7 ± 1.48 and 0 ± 0.47 for each stage in January (Table 2). During the major spawning period between October and November, the number decreased dramatically from 70 ± 33.73 to 2.33 ± 1.49 (Table 2).

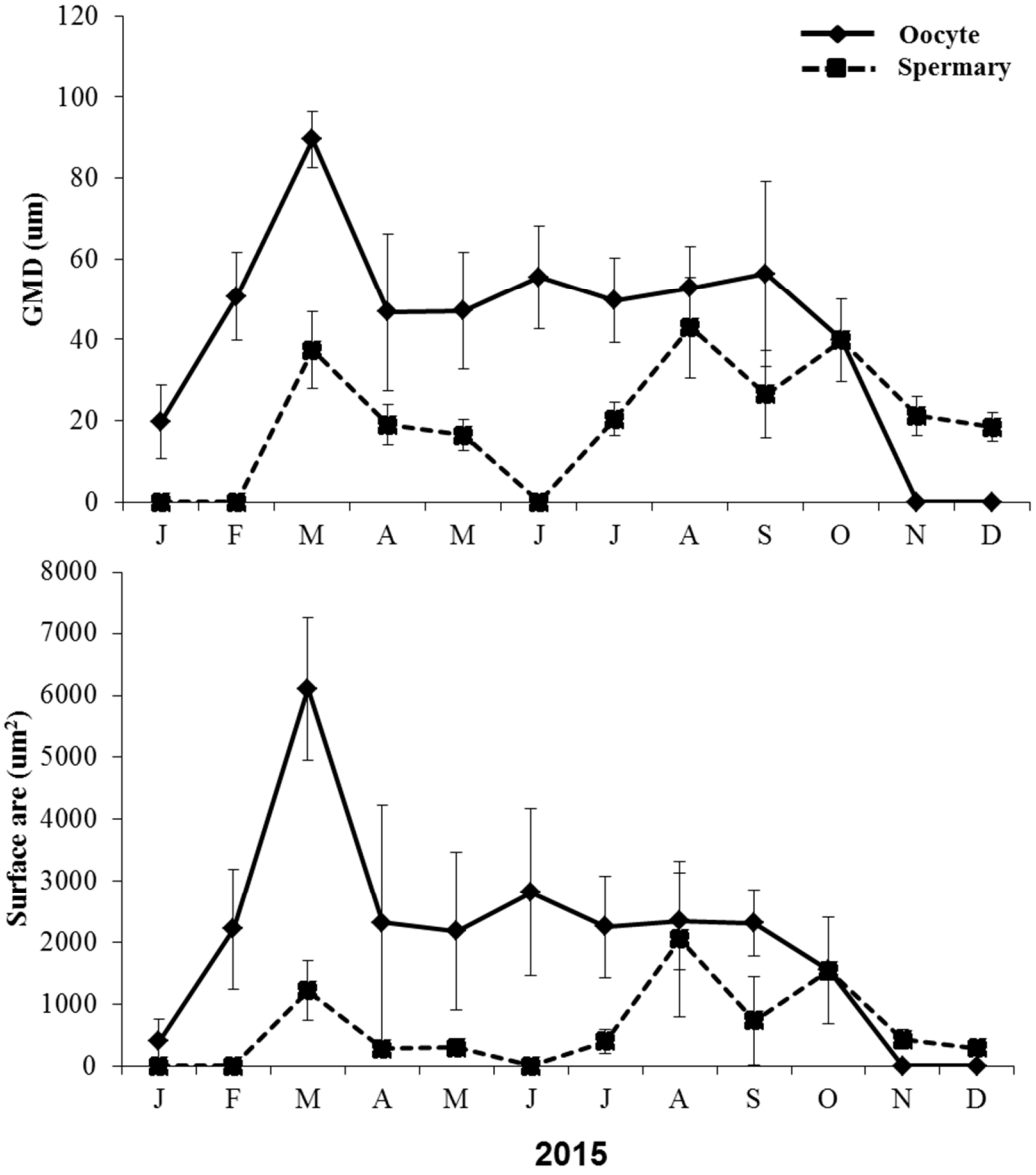

Figure 9. Seasonal changes of GMD (Geometric diameter) and surface area for both sexual gonads of *O. crispata* from the southern coast

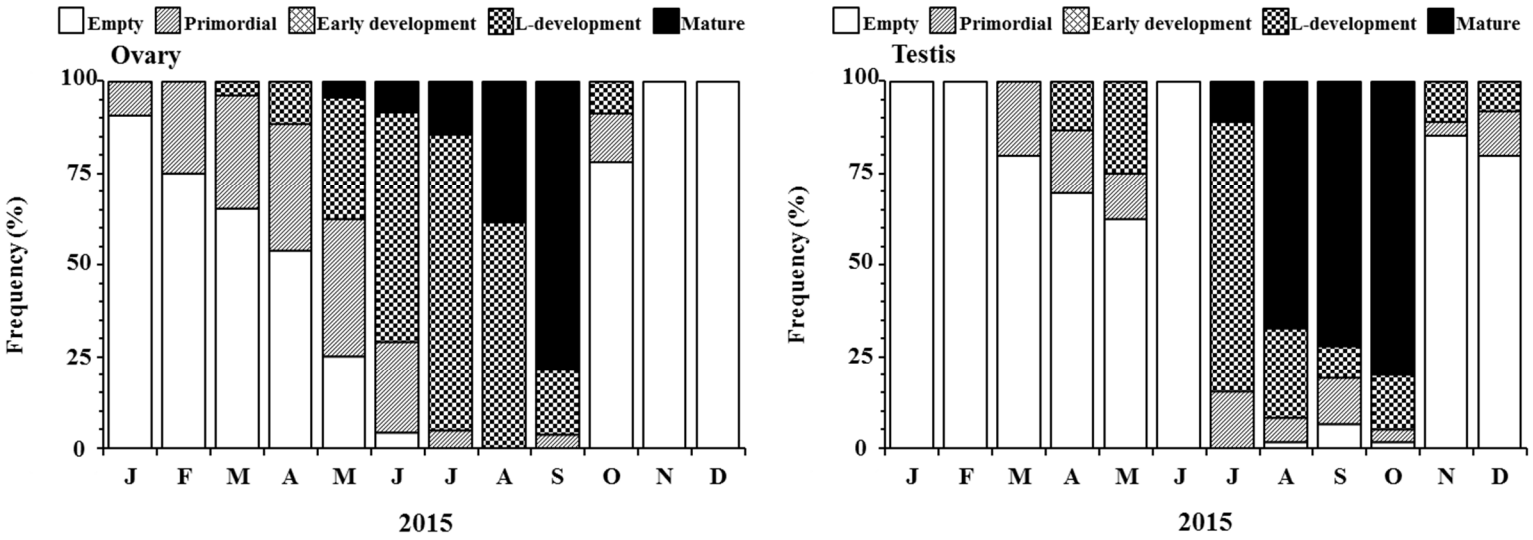

Figure 10. Percentage of *O. crispata* from the southern coast in various reproductive stages during study period

3.5. Fecundity

Number of eggs in the mesenterial filament and polyp in *A. japonica* from north and south was not significantly different (>0.05); however, in it was significantly different (<0.005) between *A. japonica* and *O. crispata* (Fig. 11. A, B). The number of eggs was higher in *A. japonica* when compared to that in *O. crispata*, but the egg size in *O. crispate* was larger when compared to that of *A. japonica.*

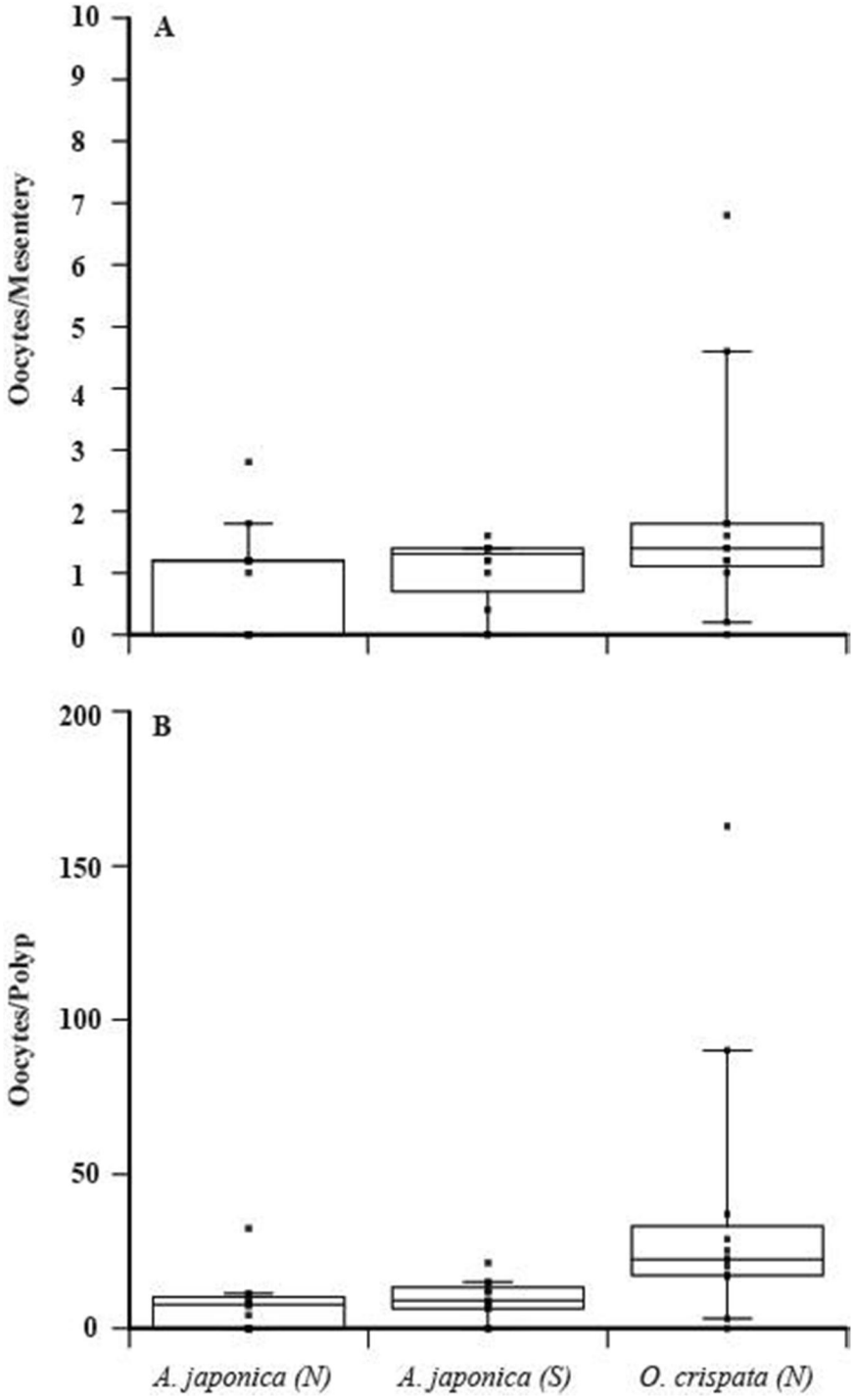

Figure 11. (A) Average mesenterial (oocyte/mesentery) fecundity and (B) polyp (oocyte/polyp) fecundity in *A. japonica* and *O. crispata*

3.6. Observation of planulae release

A total of 6 colonies of *A. japonica* and *O. crispata* were investigated for signs of planulae release. Planulae larvae were observed from *A. japonica* in August 20 and September 3, when the water temperatures were 26.7 °C and 27.3 °C at that time. The larvae released from the parent colonies possessed inherent locomotion and showed ellipsoid and spherical external features (Fig. 12, (A)). *A. japonica* colonies were bleached by extracting whole polyps from the body when releasing the larvae. A total of 9 planulae were gathered and fixed for SEM photography. The size of released planulae larvae was 538.98 ± 57.24 μm. Planulae contained numerous zooxanthellae-like under the endotherm when released (Fig. 12, (B)).

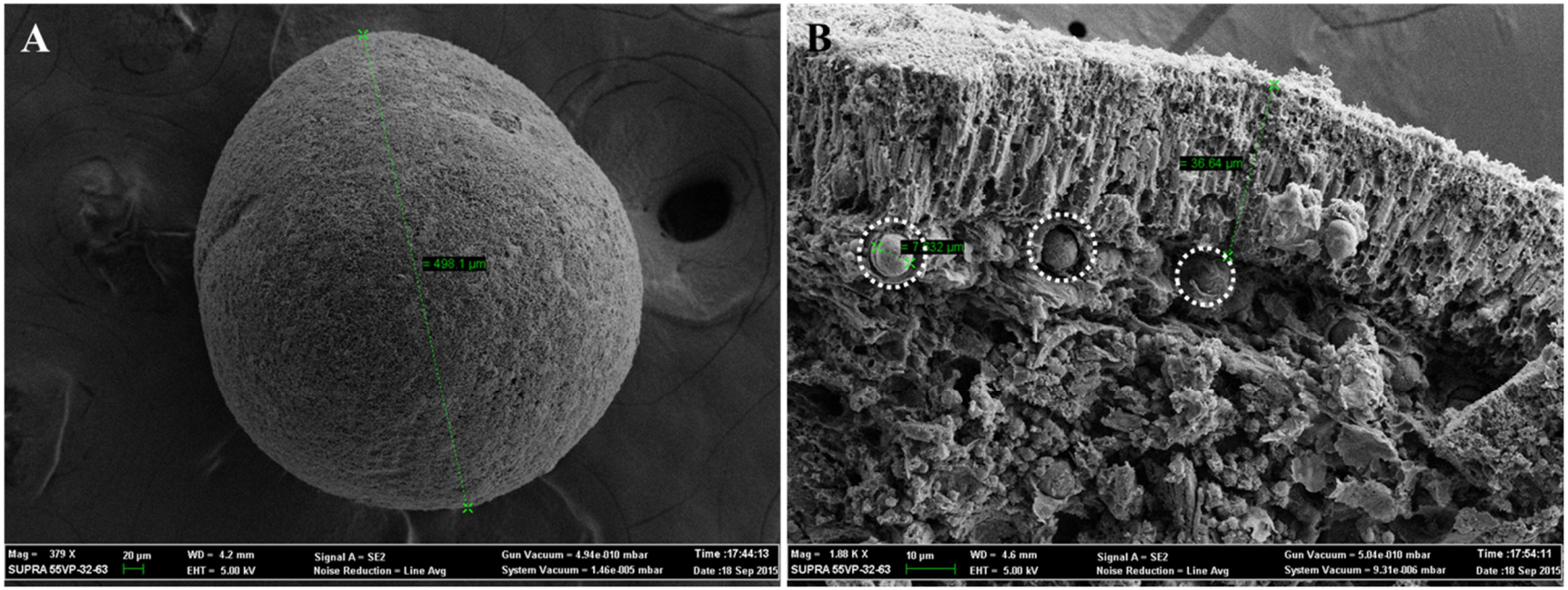

Figure 12. Collected planulae from *A. japonica* rearing in artificial aquarium. (A) Planulae SEM picture (Scale bar = 20µm). (B) Inside of planulae with zooxanthellae like spheres (Highlighted with dash circle) (Scale bar = 10µm)

Table 1. Population and biometric descriptors of *A. japonica* from north and south survey sites in Jeju Island

Age calculated based on *A. japonica* growth rate studied Jeju Island (Vieira et al. 2016), Surface area calculated by the maximum and minimum radii and height

Sampling Site	Month	Number of colonies	Height (cm)	Standard Deviation	Age (year)	Surface area (cm^2)	Standard Deviation	Sampling Site	Month	Number of colonies	Height (cm)	Standard Deviation	Age (year)	Surface area (cm^2)	Standard Deviation
North	J	5	3.99	0.41	8.3	140.59	20.05	South	J	5	1.68	0.37	3.5	37.37	17.46
	F	5	2.69	0.36	5.6	86.31	10.14		F	5	2.66	0.47	5.5	78.22	24.80
	M	5	3.42	0.50	7.1	94.69	18.08		M	4	2.12	0.21	4.4	43.79	5.20
	A	4	2.69	0.56	5.6	67.42	13.75		A	4	3.27	0.59	6.8	70.71	12.09
	M	3	3.97	0.70	8.3	115.15	14.94		M	3	2.59	0.46	5.4	83.03	26.23
	J	4	4.73	0.98	9.9	159.24	26.14		J	3	2.62	0.83	5.5	99.56	21.19
	J	3	4.67	1.32	9.7	164.68	50.29		J	2	2.64	0.46	5.5	95.32	24.40
	A	6	3.15	0.81	6.6	113.87	24.93		A	6	2.15	0.33	4.5	63.25	13.28
	S	7	2.60	0.48	5.4	73.20	13.18		S	6	2.73	0.66	5.7	81.91	22.50
	O	5	2.27	0.60	4.7	74.79	25.18		O	5	2.36	0.47	4.9	55.42	9.44
	N	5	3.80	0.92	7.9	89.25	18.85		N	4	3.04	0.42	6.3	85.68	20.16
	D	5	3.11	0.45	6.5	78.49	16.23		D	3	3.72	0.16	7.8	92.24	13.40

Table 2. Summary for sexual gonads (oocyte and spermary) of *A. japonica* and *O. crispata* variation during the research period

Polyp density calculated by counting number of polyps in 1cm^2 coral tissue, Oocyte and spermary density calculated by counting number of oocyte and speramry in 1cm^2 coral tissue. Surface area calculated by the maximum and minimum radii and height. #of polyp, oocyte and spermary calculated at the rate of total coral surface area. Ratio of sexual gonads (Female/Male) calculated by number of oocyte and speramry

Sampling site & Species	Month	Number of colonies	Polyp density	Standard deviation	Oocyte density	Standard deviation	Spermary density	Standard deviation	Surface area (cm^2)	Polyp #	Oocyte #	Spermary #	F/M
North / A. japonica	J	4	10	0.83	5	1.70	3	1.25	140.59	1448	661	380	1.7:1
	F	4	10	2.06	2	0.94	0	0.47	86.31	820	147	26	5.7:1
	M	5	7	0.49	3	0.47	2	0.47	94.69	625	256	161	1.6:1
	A	4	7	1.50	5	2.42	1	1.60	67.42	438	310	54	5.8:1
	M	3	8	3.49	3	0.63	1	1.73	115.15	956	345	115	3.0:1
	J	4	9	2.16	1	1.25	1	0.98	159.24	1433	207	127	1.6:1
	J	4	8	2.34	7	2.77	17	9.62	16.47	132	112	283	0.4:1
	A	3	9	2.35	3	1.70	15	8.16	113.87	1025	376	1731	0.2:1
	S	3	13	1.89	2	2.14	1	1.22	73.20	930	110	73	1.5:1
	O	3	6	2.16	0	0.80	0	0.00	74.79	449	30	0	•
	N	3	7	1.25	0	0.43	1	0.83	89.25	652	27	67	0.4:1
	D	3	13	2.49	1	1.22	0	0.43	78.49	997	78	20	4.0:1
South / A. japonica	J	4	12	2.86	4	3.56	2	2.16	37.37	460	149	75	2.0:1
	F	4	7	1.41	2	1.70	1	0.47	78.22	548	133	102	1.3:1
	M	4	8	2.96	3	0.94	1	0.82	43.79	328	118	44	2.7:1
	A	4	8	3.16	2	1.17	0	0.49	70.71	566	127	28	4.5:1
	M	5	6	1.33	5	5.62	1	1.10	83.03	482	374	83	1.3:1
	J	3	8	2.50	4	1.92	6	1.70	99.56	747	378	567	0.7:1
	J	4	7	0.50	3	1.25	12	11.15	95.32	620	315	1172	0.3:1
	A	4	19	2.49	3	0.80	4	1.92	63.25	1221	164	240	0.7:1
	S	3	8	1.00	2	1.25	3	2.16	81.91	655	139	246	0.6:1
	O	3	12	6.13	1	0.80	0	0.00	55.42	682	33	0	•
	N	3	10	1.70	2	0.71	0	0.00	85.68	831	171	0	•
	D	3	9	0.94	0	0.75	0	0.00	92.24	802	28	0	•
South / O. crispata	J	4	7	1.48	3	2.94	0	0.47					9.1:1
	F	4	5	0.83	2	0.47	0	0.00					•
	M	5	7	0.89	3	0.94	1	0.47					4.0:1
	A	4	4	0.87	2	1.89	0	0.00					•
	M	4	6	1.22	3	1.92	2	1.33					1.3:1
	J	3	6	0.94	3	2.15	1	1.30					2.1:1
	J	3	5	0.82	3	1.73	1	0.83					2.0:1
	A	3	8	0.94	33	28.06	4	2.18					9.5:1
	S	3	7	0.47	17	8.64	2	1.48					7.7:1
	O	3	9	2.36	0	0.00	70	33.73					•
	N	3	8	2.16	0	0.00	2	1.49					•
	D	3	8	0.82	0	0.00	1	0.87					•

4. Discussion and Conclusion

The results from the present study demonstrated that *A. japonica* and *O. crispata* in Jeju Island are maintaining local population through sexual reproduction with annual gametogenesis. For both species *A. japonica* and *O. crispata*, sexual reproduction patterns and seasonality agree with the previous observations of Harii et al (2001) and Igarashi et al (1992) from Japan, and Nakano & Yamazato (1992) from Okinawa in Japan and Lam (2000) in Hong Kong, China. *A.japonica* is a hermaphroditic brooding species with oocytes and spermaries developing within separate mesenteries of the polyps. *O. crispata* is simultaneous hermaphroditic specie, and the gonadal arrangement is different with *A. japonica* that oocytes and spermaries developing within same mesenteries (Clark 1997, Collinson 1997, Lam 2000).

The reproductive cycle of *A. japonica* at North and South appeared to be annual with oogenesis requiring about 8 months, a reproductive pattern coincide with the corals worldwide represented (Harrison and Wallace 1990). Oogenesis period of *A. japonica* in Jeju shorter in duration to *A. japonica* in Japan, which takes 10 ~ 11 months (Harii et al 2001). Oogenesis of *O. crispata* with duration of 8 months and spawning was observed in September, coincided with the pattern observe in Hong Kong. For *A. japonica* from both sites, the number of oocytes was relatively more than spermary before the spawning season and the number of spermary turn over just after the spawning periods (Table 2). Our interpretation based on the ratio of female and male and the frequency of reproductive stages indicated that the spawning activities of oocytes were relatively finished in late August one month earlier than speramries. In winter when the spawning was finished, the number of oocyte gradually was increased for preparing for next spawning in next year. For the spawning activity occasions, major fertilizations of *A. japonica* were occurred in late August when the spawning periods of oocytes and speramraies were overlapped. However,

fertilizations of *O. crispata* were occurred in late September one month later than the *A. japonica*.

We hypothesized the *A. japonica* in north and south have different spawning periods due to the difference of SST in expected spawning periods but the periods at both sites appear in late August when the surface sea water temperature reached a maximum as 24.01 ± 1.37 ~ 25.02 ± 1.52 °C with same maturation periods. According to previous coral reproductive studies at Okinawa and Tokyo bay in Japan, the spawning periods of *A .japonica* occurred when the surface seawater temperature reached maximum and just after maximum as ranged 23 ~ 27 °C (Harii et al. 2001). In this study also, major spawning activity occurred when the sea water temperature at the highest. In addition, spawning period of *O. crispata* from south appears in September and October when the surface seawater temperature 24.95 ± 0.79 ~ 25.02 ± 1.52 °C. In Hong Kong, China, *O. crispata* had spawning period during July to October when the SST ranged as 25 ~ 27 °C (Lam 2000). Although gap of SST between Japan, Hong Kong and Jeju Island could be a reason behind the difference in reproductive periods for the coral species, the real causes is most likely a complex combination of various environmental factors including, however, the SST was basic and important standard for ecological observation (Kang 2010; Viera et al. 2016). In addition, one year observation could be obtain partial of reproductive patterns, however, only long-term monitoring will be able to completely understand reproductive studies for two corals, *A. japonica* and *O. crisptata*.

In conclusion, *A. japonica* and *O. crispata* have seasonal patterns of gametogenesis, with gonial mitosis commencing predominantly in January and the major spawning of these two species appeared when the surface seawater temperature ranged 24.01 ± 1.37 ~ 25.02 ± 1.52 °C in August and September in Jeju Island. The GMD between north and south *A. japonica* is not significantly different, however, when compared to *O. crispata*, the GMD of *O. crispata* is less. Due to the GMD of *A. japonica* is larger than *O. crispata*, the number of oocytes per mesentery and polyp in *A. japonica* is less than that in *O. crispata* which is

larger eggs, less number (*A. japonica*) and smaller eggs more number (*O. crispata*). In addition, planula releasing experiment, the present study revealed that *A. japonica* is a hermaphroditic brooding species through histology and observing its larvae possessed zooxanthelate like in their body. In addition, reproductive pattern of *O. crispata* was establish as a hermaphroditic species but could not distinguish the reproductive mode in the present study. In this respect, *A. japonica* distributed in Jeju is hermaphroditic brooding specie. According to Nakano & Yamazato (1992) study, *O. crisptata* can be both a broadcast spawner and a brooder through releasing eggs and sexual planulae (without zooxanthellate) and asexual planulae (with zooxanthellae) depending on the environmental conditions, however we could not confirm either in our study. Harriott (1992) mentioned that brooding species have an advantage in isolated reef systems due to the coral larvae can settle and maintain their population in a short time just after releasing from the parental colonies. This could be the reason for increase in the population of *A. japonica* in Jeju for past couple decades. The present study was carried out for one year and from two sampling sites giving us the basic information of gametogenesis, however for more precise determination of coral reproduction patterns and mode requires long-term observations and more continuous sampling within large number of population at the species level.

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