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# Seasonal Changes in Gonads of Crown-of-thorns Starfish, *Acanthaster planci*

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## Abstract

The crown-of-thorns starfish, *Acanthaster planci*, is a large species of the Asteroidea. Corresponding with its size, a huge number of gametes are produced in the gonads of each individual and are spawned during the breeding season. This study examined histological seasonal changes in the ovaries and testes of *A. planci* inhabiting a coral reef near Sesoko Island, Okinawa Prefecture, Japan. In July, *A. planci* ovaries and testes were large and filled with fully grown oocytes and mature spermatozoa, respectively. In contrast, both ovaries and testes shrank significantly in August. Residues remained in the empty ovaries and a few spermatozoa were present in the small testes. This suggests that gamete shedding in Okinawan *A. planci* occurs in July and August. In October, oogonia and developing oocytes were found at the periphery of the ovary. Spermatogonia and spermatocytes were also observed in the testis. Although the sizes of gonads were still small in January and February, the numbers of oogonia and developing oocytes increased at the periphery of the ovaries, and spermatogenesis occurred actively in the lumen of the testes. After April, oogenesis in ovaries and spermatogenesis in testes were activated remarkably. Together, these results suggest that eggs and spermatozoa are freshly prepared in ovary and testis of *A. planci* following an annual cycle.

Keywords: Crown-of-thorns starfish (*Acanthaster planci*), ovary, testis, gametogenesis; histological studies

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## 1. Introduction

The crown-of-thorns starfish, *Acanthaster planci*, inhabit coral reefs in the Indo-Pacific regions. This is a large starfish of the Asteroidea and has more than 15 arms. Because there is a pair of gonad in each arm, an individual *A. palnci* possesses about thirty ovaries or testes. During the breeding season, a huge number of gametes are spawned from each starfish. The bipinnaria and brachiolaria larvae feed on single-celled algae of the phytoplankton. Although the infant *A. planci* after metamorphosis feeds on the coralline-algae surface, juvenile *A. planci* increase their arm numbers from 5 to about 15 and begin feeding on hard coral (Yamaguchi, 1974). Adult *A. palnci* feeds on the living surface tissue of hard corals, leaving dead white coral skeletons cleared of tissue on the surface of the corals (Yamaguchi, 1974; Birkeland and Lucas, 1990; Lucas, 2013).

*A. planci* are blamed for extensive coral mortality in a large number of coral reefs (Birkeland and Lucas, 1990; De'ath *et al*., 2012; Fabricius *et al*., 2010; Nakamura *et al*., 2014). Once an unpredictable primary outbreak occurs, the large population of adult *A. planci* produces enormous amounts of eggs, which lead to secondary outbreaks (Kenchington, 1977). In Okinawa Island, Japan, the first recorded observation of high-density populations of *A. palnci* occurred in 1969 (Nishihira and Yamazato, 1972). Since the early 1970s, *A. planci* control programs have started to protect the reef-building coral at several important fishing grounds and tourism sites in Okinawa Island (Yamaguchi, 1986). Until the early 2000s, more than ten thousands *A. planci* were removed from the reefs of Okinawa every year (Nakamura *et al*., 2014). Despite these huge efforts, there are still large *A. planci* populations in Okinawa.

In starfish, gamete release is triggered by a relaxin-like gonad-stimulating peptide (RGP) secreted from radial nerve cords (Mita *et al*., 2009). RGP acts on gonads to produce the second mediator, 1-methyladenine (1-MeAde) which is the maturation-inducing hormone (MIH) of starfish (Kanatani *et al*., 1969; Kanatani, 1985). 1-MeAde is produced by ovarian follicle cells around oocyte in female (Hirai and Kanatani, 1971; Hirai *et al*., 1973) and testicular interstitial cells in male (Kubota and Kanatani, 1977). Recently, we identified the chemical structure of RGP in *A. planci* (Mita *et al*., 2015). Synthetic RGP could induce oocyte maturation and ovulation in ovarian fragments of *A. planci*. This strongly suggests that the reproduction in *A. planci* is regulated by RGP as a gonadotropin. Although there are many literatures about *A. planci* reproduction (Lucas *et al*., 1973; Birkeland and Lucas, 1990; Babcock and Mundy, 1992), little is known about histological seasonal changes in the gonads of *A. planci* inhabiting Okinawa. Here, to elucidate the reproductive cycle in gonads of *A. planci*, we carried out histological observations of ovaries and testes of *A. planci* throughout the year.

## 2. Materials and Methods

### 2. 1 Collection of *A. planci* specimen

Because *A. planci* specimen larger than 20 cm in diameter is sexual matured in the wild (Birkeland and Lucas, 1990), the fully matured specimens (approximately 24 ~ 28 cm in diameter) were collected from a coral reef near Sesoko Island, Okinawa Prefecture, Japan (26º38N, 127º52E) every one or two months from December 2009 to November 2010. Numbers of female and male from each sampling used for histological observations were summarized in Table 1.

Table 1. Numbers of fully matured specimens of the crown-of-thorns starfish *Acanthaster planci* collected from a coral reef near Sesoko Island, Okinawa from December 2009 to November 2010

| Dates | Number | | |
|---|---|---|---|
| | Female | Male | Total |
| 11-Dec | 7 | 4 | 11 |
| 25-Jan | 2 | 5 | 7 |
| 26-Feb | 5 | 1 | 6 |
| 18-Mar | 4 | 2 | 6 |
| 20-Apr | 5 | 1 | 6 |
| 21-Jun | 5 | 4 | 9 |
| 20-Jul | 3 | 3 | 6 |
| 20-Aug | 4 | 1 | 5 |
| 25-Oct | 4 | 7 | 11 |
| 26-Nov | 4 | 4 | 8 |
| Total | 43 | 32 | 75 |

### 2. 2 Sample preparation and histological observation

For histological observations, pieces of ovaries and testes were fixed in Bouin's solution. The specimens were dehydrated through a graded ethanol series, embedded in paraffin, and then sectioned with a thickness of 7 μm. After

staining with haematoxylin and eosin, the sections were examined using an Olympus BX50 light microscope equipped with an Olympus DP70 digital camera system.

## 3. Results

### 3. 1 Observation of ovaries

In December (Figs. 1a and A) and January (Figs. 1b and B), four ovaries out of nine individuals contained a few large developed oocytes in the central area of the lumen. Some young germ cells (10μm in diameter) such as oogonia and oocytes at the chromatin nucleolus stage and peri-nucleolus stages were localized to the periphery of the lumen. Some degenerating and large, developed oocytes were observed in the central area of the lumen in the remaining five ovaries. These ovaries also contained oogonia and young oocytes at the inner periphery of the lumen (Fig. 2A).

In February (Figs. 1c and C) and March (Figs. 1d and D), seven ovaries out of nine individuals had many large developed oocytes in the lumen. However, the oocytes in five of these ovaries were degenerating, whereas the remaining two ovaries had only young oocytes at the inner periphery of the empty lumen. Also, clusters of degenerating oocytes enclosed by many presumed phagocytes were observed in the central part of an ovary (Figs. 1d and D).

In April (Figs. 1e and E), June (Figs. 1f and F) and July (Figs. 1g and G), a large amount of fully-grown oocytes (120−140μm in diameter) were distributed in the lumen of ovaries in all thirteen females. The cytoplasm of the oocytes was strongly stained with eosin. Some young oocytes stained with haematoxylin were distributed at the periphery of the lumen (Figs. 1e and E).

In August (Figs. 1h and H) and October (Figs. 1i and I), one ovary out of eight individuals had many developed

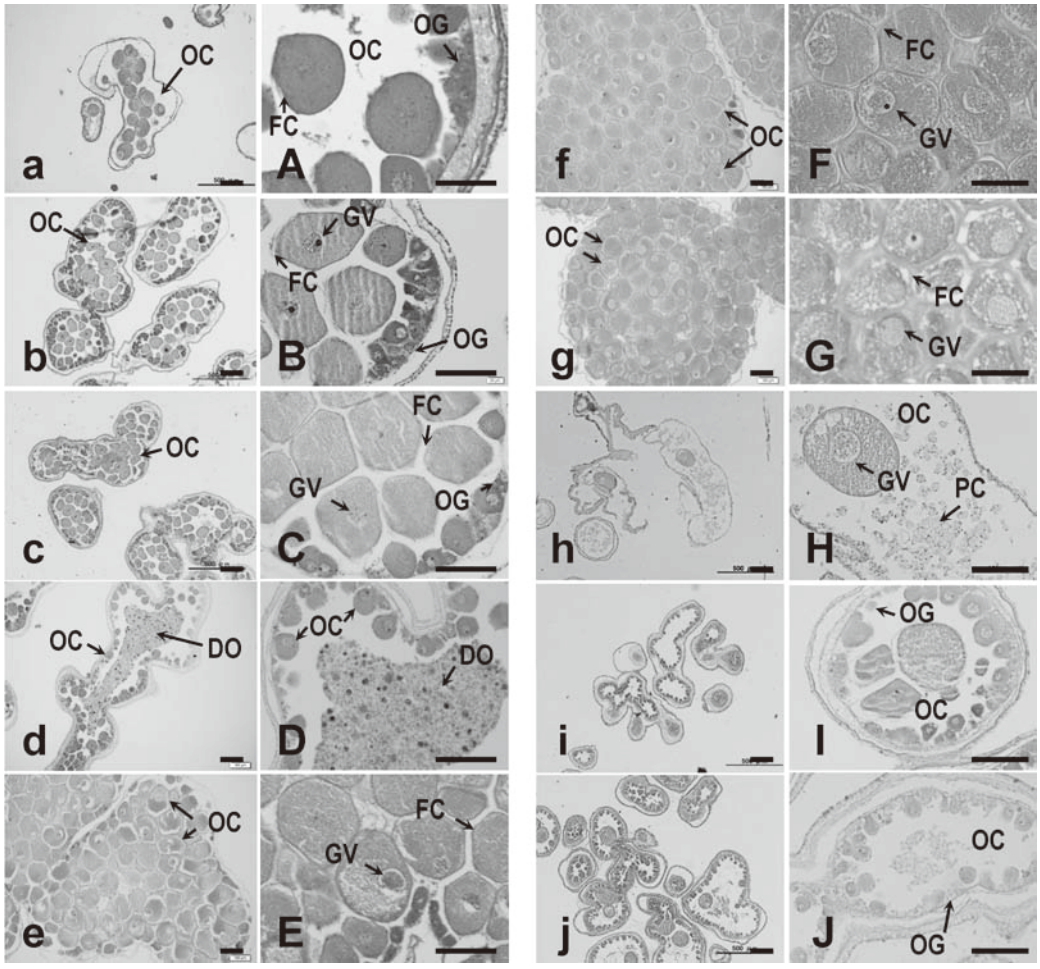


Figure 1. Histological sections of the ovary of the crown-of-thorns starfish *Acanthaster planci* collected in December (a, A), January (b, B), February (c, C), March (d, D), April (e, E), June (f, F), July (g, G), August (h, H), October (i, I), and November (j, J). Bars show 100 μm. Abbreviations: DO, degenerating oocytes; FC, follicle cells; GV, germinal vesicle; OC, oocyte; OG, oogonia; PC, phagocyte.

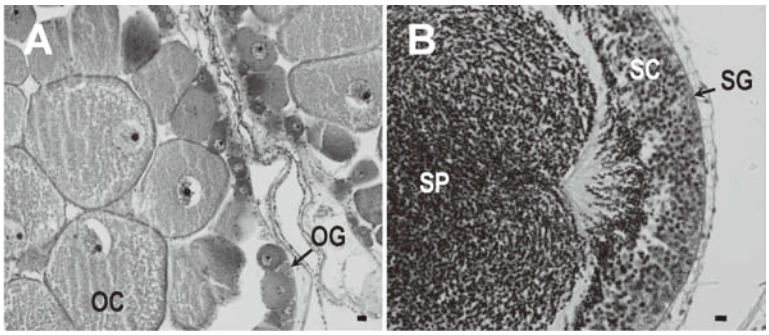


Figure 2. Histological sections of the ovary (A) and testis (B) of the crown-of-thorns starfish *Acanthaster planci* collected in January. Bars show 10μm. Abbreviations: OC, oocyte; OG, oogonia; SC, spermatocyte; SG, spermatogonia; SP, spermatozoa.

oocytes in the lumen, although the other seven ovaries had less-developed oocytes or an empty lumen. In addition, there were a few young oocytes observed at the periphery of the lumen.

In November (Figs. 1j and J), a large number of fully-grown oocytes were observed in the central region of the lumen in two out of four females. However, the cytoplasm of most of the oocytes had altered shapes, indicating degeneration. Some clusters of degenerating oocytes were also observed in the lumen. The remaining two ovaries had not developed oocytes at all and only displayed young oocytes at the periphery of the lumen.

### 3. 2 Observation of testes

In December (Figs. 3a and A) and January (Figs. 3b and B), spermatozoa in the central region of the lumen were either absent or present in low amounts. Whereas seven testes out of nine individuals were almost or entirely empty, spermatozoa were found in large amounts in the remaining two testes. A thin layer of spermatogenic germ cells consisting of spermatogonia and spermatocytes was found lining the inner periphery of the lumen (Fig. 2B).

In February (Figs. 3c and C), March (Figs. 3d and D) and April (Figs. 3e and E), all testes of four individuals had large amounts of spermatozoa in the lumen, whereby a thick layer of spermatogenic germ cells covered the inner periphery of the lumen.

In June (Figs. 3f and F), July (Figs. 3g and G) and August (Figs. 3h and H), all seven testes were large in size, and large amounts of spermatozoa filled the central part of the lumen. A very thin layer of spermatogenic germ cells covered the inner periphery of the lumen.

In October (Figs. 3i and I) and November (Figs. 3j and J), spermatozoa were found in extremely reduced amounts and numbers in eight testes out of eleven individuals. The remaining three testes had large amounts of spermatozoa in the lumen. A thin or thick layer of spermatogenetic tissues covered the inner periphery of the lumen, regardless of the amount of spermatozoa.

## 4. Discussion

In this study, ovaries and testes of *A. planci* inhabiting a coral reef near Sesoko Island in Okinawa were examined histologically throughout the year. Young female germ cells such as oogonia and oocytes were only distributed at the inner periphery of the lumen in the ovaries of *A. planci*. However, large and developed oocytes were only distributed in the central lumen of the ovaries. This observation indicates that early oogenesis proceeds at the inner periphery of the ovary, and that developed oocytes aggregate and complete their development in the center of the lumen. Young oocytes increased in number at the periphery of the ovaries in January and February. It thus seems likely that oogenesis starts in the ovaries around January and February. Presumably, oogenesis in Okinawan *A. planci* is activated after March.

In contrast, active spermatogenesis occured within a layer of spermatogenic cells consisting of spermatogonia and spermatocytes. These cells were observed at the inner periphery of the testes in December. This layer gradually increased in thickness during January, February and March. A thicker layer was found in the testes in April. Between June and August, the layer of spermatogenic germ cells in the testes decreased in thickness or disappeared altogether. A thin layer of spermatogenic germ cells reappeared in the testes in October and November. These observations strongly suggest that active spermatogenesis starts mainly in February and March, and it peaks in April.

Furthermore, spermatozoa were found to be stored in the

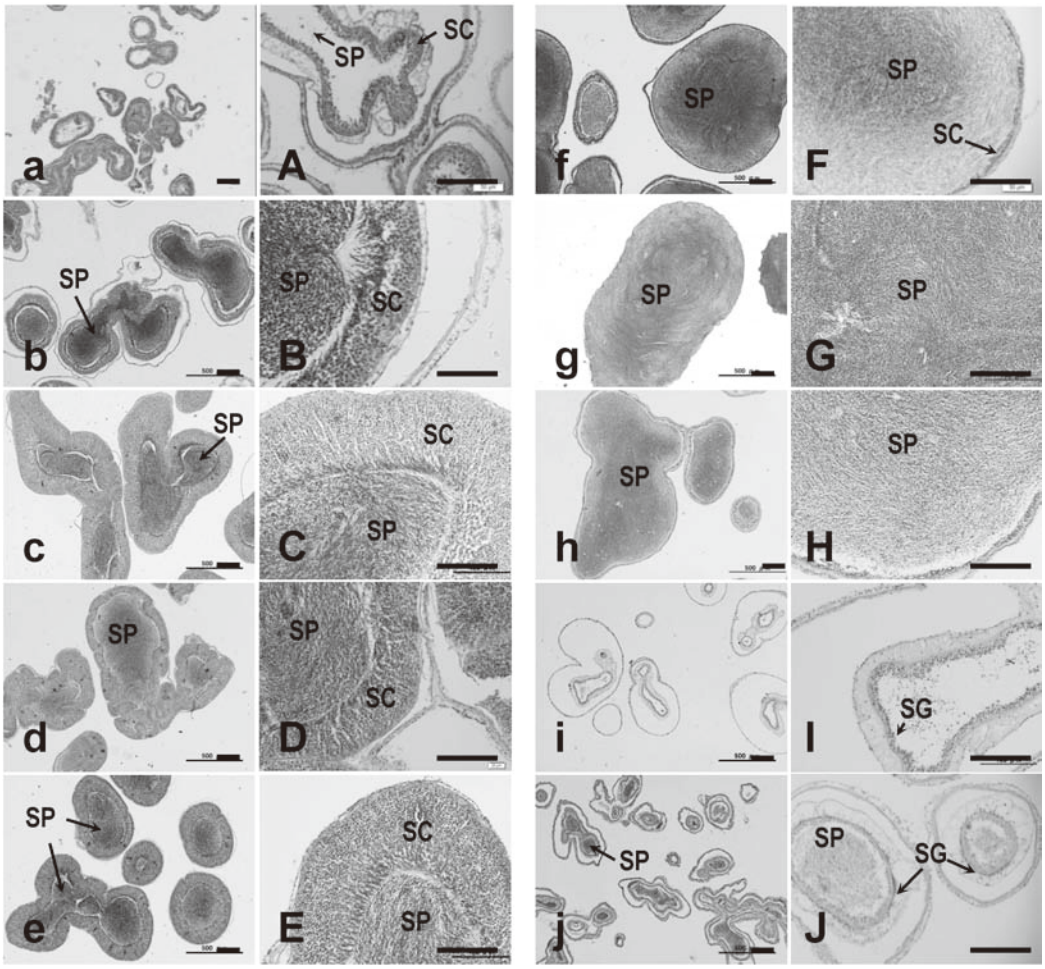


Figure 3. Histological sections of the testis of the crown-of-thorns starfish *Acanthaseter planci* collected in December (a, A), January (b, B), February (c, C), March (d, D), April (e, E), June (f, F), July (g, G), August (h, H), October (i, I), and November (j, J). Bars show 100 μm. Abbreviations: SC, spermatocyte; SG, spermatogonia; SP, spermatozoa.

central lumen of the testes in January and February. Large numbers of spermatozoa were stored in the lumen of the testes from March through August. Spermatozoa in the lumen of some testes first disappeared in October. In addition, large and developed oocytes increased in number in the central area of the ovaries in April and June and then suddenly disappeared or decreased in number in some individuals in August and October. Based on these observations, it was concluded that spawning occurred in Okinawan *A. planci* from July to August. This agrees with observations in previous studies (Yamazato and Kiyan, 1973; Yokochi and Ogura, 1987; Yasuda *et al*., 2010). However, matured oocytes and spermatozoa remained in the ovaries and testes, respectively, in November after spawning. Most of the developed oocytes in the ovaries degenerated. On the contrary, it was difficult to confirm the degeneration of spermatozoa. These histological characteristics strongly suggest that the mature eggs, that failed to release, remain within the ovaries and degenerate after the spawning season. They are never alive until the next breeding season.

Pastor-de-Ward and coworkers have reported in the starfish *Cosmasterias lurida* that the nutritive phagocytes appear within gonads just before gametogenesis (Pastor-de-Ward *et al*., 2007). In contrast, nutritive phagocytes were not observed in *A. planci* gonads. Thus presumably, a precursor for yolk protein is contained in pyloric caeca of *A. planci*, in the same manner as *Asterias rubens* (Schoenmakers *et al*., 1981), *Asterina stellifera* (Carvalho and Ventura, 2002), *Echinaster sepositus* (Riesgo *et al*., 2011) and *Pharia pyramidatus* (Benítez-Villalobos and Martínez-Garcia, 2012).

This study showed that both ovaries and testes in Okinawan *A. planci* were sufficiently developed and filled with fully grown oocytes and spermatozoa until June. Previous studies have shown that the timing of spawning of *A. planci* is closely correlated with water temperature and that the spawning begins at around 28ºC (Yasuda *et al*.,

2010). Because RGP is a first trigger for gamete shedding (Mita *et al*., 2009), it seems likely that RGP is secreted from radial nerve cords in *A. planci*, when water temperature exceeds 28°C. The RGP secretion in *Patiria* (= *Asterina*) *pectinifera* is closely correlated to an increase upon increase in intracellular $Ca^{2+}$ concentration (Mita, 2013). This suggests that *A. planci* RGP is secreted from the radial nerve cords as increasing in intracellular $Ca^{2+}$ concentrations, although the mechanism linking temperature and $Ca^{2+}$ content remains elusive.

RGP is functionally analogous to the vertebrate luteinizing hormone (LH), especially piscine and amphibian LHs, acting on the ovarian follicle cells to produce MIH which induces the final maturation or meiotic resumption of the oocyte (Nagahama *et al*., 1995). This suggests strongly that reproduction in *A. planci* depends on RGP. Without RGP, gamete shedding does not occur. It may also be possible that disturbance of RGP synthesis or secretion brings about shift the timing of gamete maturation and disruption of the reproductive cycle in *A. planci*. Further studies on the regulatory mechanism of RGP could provide useful insights into a new technique for reproductive control in *A. planci*.

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# オニヒトデ生殖巣の経年変化

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生命科学分野

**要　旨**

沖縄県瀬底島周辺に生息するオニヒトデを定期的にサンプリングし，生殖巣を組織学的に観察した。その結果，卵巣・精巣とも 3 月から 6 月にかけてもっとも著しい成長が見られ，8 月以降は極端に委縮していた。このことからオニヒトデの繁殖期は 6 月末から 8 月初めであることが示唆された。また，卵や精子は，毎年，生殖巣内で新規に調整されることも明らかになった。

**キーワード:** オニヒトデ，卵巣，精巣，卵子形成，精子形成

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