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Reproduction in the Apodid Sea Cucumber *Patinapta ooplax*: Semilunar Spawning Cycle and Sex Change

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ABSTRACT—Many fertile individuals of the apodid holothurian *Patinapta ooplax*, living in the intertidal area of the Ichiki Fishing Port in southern Kyushu, Japan, spawned during the two days after every full and new moon, probably the first and second days, in the period from the middle of July to the end of August during 1990, 1992 and 1993. Matured individuals were divided into three sexual types: males, hermaphroditic males with an early stage of oocytes, and females, using a dissecting microscope. The distribution frequency and gonadal histology of these sexual types indicate that some individuals changed from male to female or in the reverse direction at two-week intervals between spawnings, and suggest that some change first from male to female, and then back to male during the main breeding season. In addition, it was found that during the main breeding season, synchronous gametogenesis occurred in association with the sex changes, and that the period from the initiation of spermatogonia proliferation to sperm release during the same season was two weeks, and the period from the initiation of oocyte growth to egg shedding was probably slightly longer than two weeks.

INTRODUCTION

In free-spawning marine invertebrates, spawning synchrony is the most fundamental requirement for maximizing fertilization, and spawning synchrony has been known to be correlated with changes in a variety of environmental factors; for example, temperature, phases of the moon, illumination, presence of phytoplankton (reviewed by Giese and Kanatani, 1987). Among echinoderms, spawning associated with the definite moon phases has been observed in the following species: the crinoid *Comanthus japonica* (Dan and Kubota, 1960; Kubota, 1980), the echinoids *Diadema setosum* (Fox, 1923; Pearse, 1990), *D. antillarum*, *D. mexicanum*, *Eucidaris tribuloides*, and *Lytechinus variegatus* (Lessios, 1991), *Centrostephanus coronatus* (Kennedy and Pearse, 1975), *Mespilia globulus* (Kobayashi, 1967). Recently, the holothuroids *Bohadschia argus*, *Euapta godeffroyi*, *Stichopus chloronotus*, and *S. variegatus* joined this group based on the investigations on the Great Barrier Reef by Babcock *et al.* (1992) and *Polycheira rufescens* by Kubota and Tomari (1998).

Two patterns of sex change have been observed in echinoderms; protandric and reciprocal changes. As protandric species, ten [four asteroids, three holothuroids, and three ophiuroids] were cited by Polikansky (1982) and in addition, four [one asteroid, one holothuroid, and two ophiuroids] by Sewell (1994). Another type, the reciprocal sex change, was found in the holothuroid *P. rufescens*, where sex converted from male to female or in the inverse direction during semilunar

intervals between spawnings (Kubota and Tomari, 1998).

This paper reports that the apodid holothurian *Patinapta ooplax* spawned during certain moon phases and reciprocally changed sex during the intervals between spawnings, and that these modes of spawning and sex change closely resemble those of the apodid *P. rufescens*.

MATERIALS AND METHODS

Patinapta ooplax is distributed in the southern half of Japan including Okinawa and China, and similar in external dimensions to the wide-spread apodid *Leptosynapta inherens*, although *P. ooplax* is smaller in size (Uchinoumi, 1979). Collections were conducted in 1990, 1992 and 1993 at the Ichiki Fishing Port, Kagoshima, Kyushu, Japan (31°41'29"N, 130°17'20"E). This site is in the intertidal area of the estuary common to the Yafusa River and Osato River (Fig. 1), and its substratum is composed of sand with a small proportion of mud. The sea cucumbers were obtained at low tide by digging out those lying in an elongated form in the substratum of 5 to 10 cm depth. They were placed with the surrounding sand in a small vessel, and transported to the laboratory in ice-cold conditions. They were then placed in 9 cm Petri dishes containing seawater, and both the complete individuals with the head and tail and the anterior pieces with the head and gonad were selected out under a dissecting microscope. The anterior piece appears to be produced by autotomy instantly induced by a forced body-stretch during digging. Using the complete individuals and the anterior pieces, the sex and maturity were judged by examining gonads. The anterior body-wall was cut longitudinally, and the gonad that protruded were observed under a dissecting microscope. The outer membrane of the gonad is so thin and transparent that the underlying reproductive cells were clearly visible. Before sex determination some complete individuals were dipped in tap water, and then their sizes were measured, because the length and width of sea cucumbers are known to be nearly constant in tap water. The complete individuals were also used for determining the

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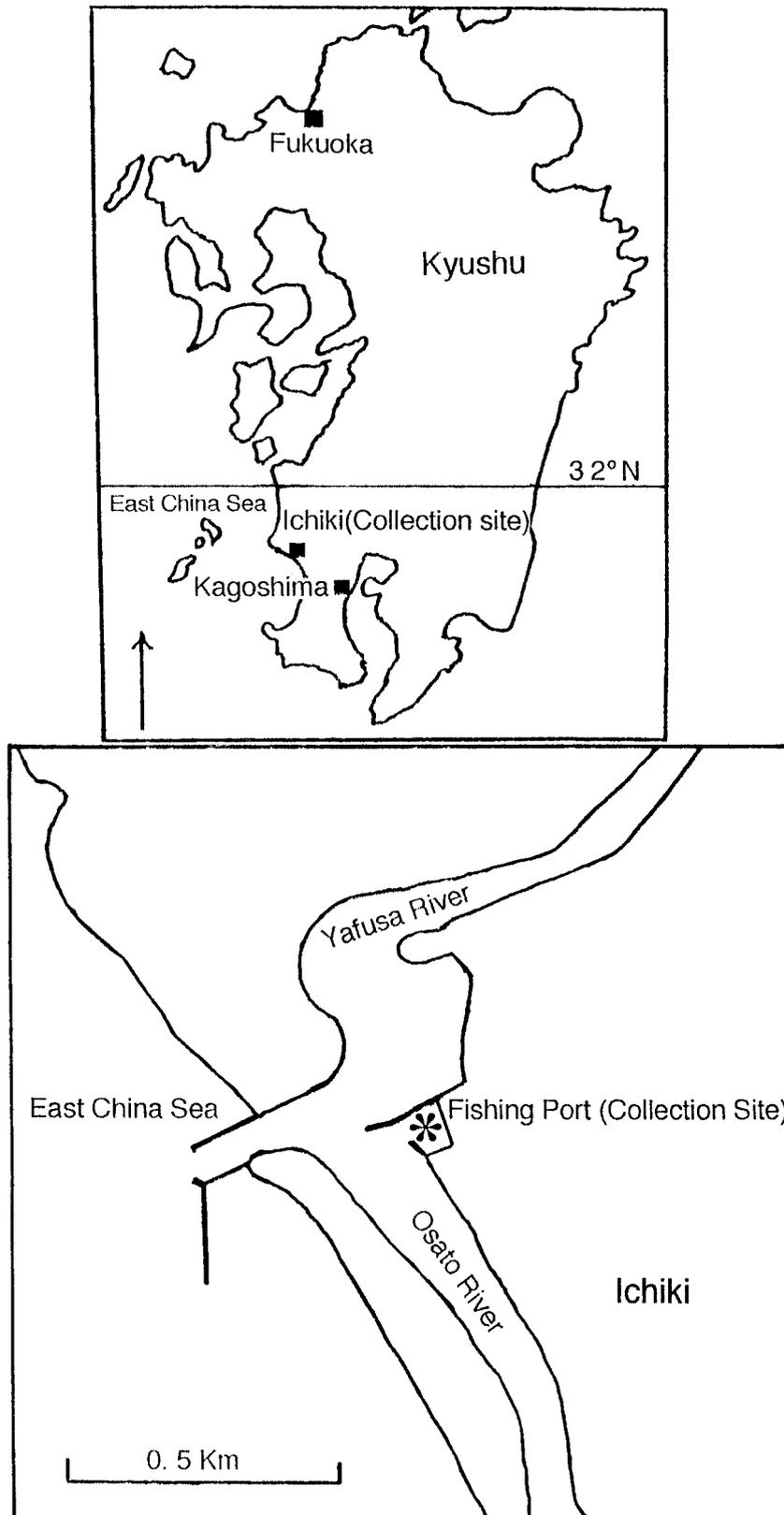


Fig. 1. Map showing location of the collection site, Ichiki Fishing Port (asterisks). Upper: the location of Ichiki in Kyushu region. Ichiki is south from latitude 32°N, and faces the East China Sea. Lower: enlarged Ichiki area, showing the location and dimensions of Fishing Port.

gonad index. In these studies, for convenience, the following formula was adopted for calculating the gonad index, because in most cases the gonad dry-mass of a single individual for the reproductive resting period was less than 0.1 mg.

$$\text{gonad index} = \frac{\text{Sum of gonad dry-masses of all specimens used}}{\text{Sum of body-wall dry-masses of the same specimens}} \times 100$$

The procedures for preparing the dry masses of the gonad and the body wall were described elsewhere (Kubota and Tomari, 1998).

The methods of the gonadal histology is the same as in the case of *P. rufescens* (Kubota and Tomari, 1998): in brief, the gonadal tubules of the precold sea cucumber were excised and fixed with 10% formalin seawater on ice, and stored in the fix solution at room temperatures until sectioning. The tubules were post-fixed in Bouin fluid, embedded in paraffin, sectioned 4–6 μm thick, stained with haematoxylin-eosin, and observed under a microscope.

RESULTS

The material used in these studies of *P. ooplax* was found dispersed in the substratum of the Ichiki station at low tide throughout the year; 45 individuals were collected in an area of 2 m² immediately before the beginning of spawning in the second half of July 1993. The sea cucumber was small in size; individuals collected in August 1994 were on average 4.2 \pm 0.54 cm long and 5.3 \pm 0.54 mm wide in tap water. The gonad consisted of paired tufts of many tubules. The change in gonad size for a year is shown in Fig. 2 as that of the gonad index. The unimodal curve of the graph indicates that the breeding season of this species is once a year and in the summer.

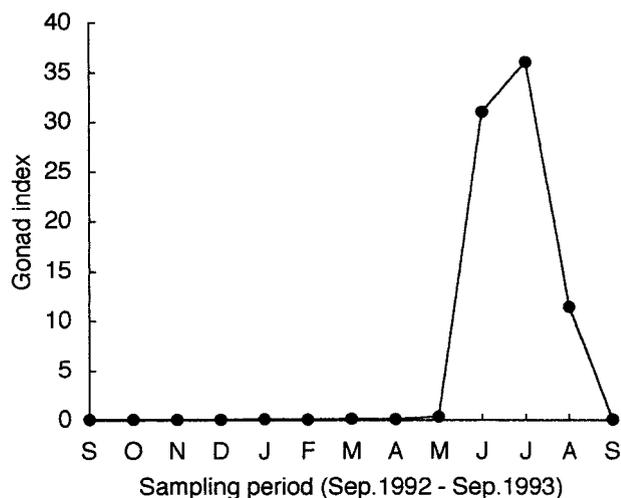


Fig. 2. Annual change in the gonad index. Values were obtained as [sum of dry gonad masses/sum of dry body-wall masses of all specimens used $\times 100$]. N = 9 to 15.

Gamete maturation and release

The investigation concerning gamete maturation and release of *P. ooplax* was conducted in 1990, 1992 and 1993. Examination of the collected specimens showed that spawning occurred in a part of the population during the period earlier than the middle of July. That is, the ratio of individuals with

the spent gonad to fertile individuals was low [0% (0/58 individuals in the total of the following four days) on 27 June, and 5, 8 and 9 July, and 46% (12/26) on 12 July (the fourth day after full moon, +4d) in 1990; 7% (1/15) on 22 June, and 0% (0/29) on 4 July (+1d) in 1993]. In these cases, fertile individuals contain both individuals with such reproductive cells as spermatocytes, sperm, and well developed oocytes, and with a spent gonad. Similarly, the ratio of individuals with sperm was low [20% (1/5) on 27 June, and 13% (2/15) on 9 July in 1990; 13% (2/15) on 22 June, and 3% (1/29) on 4 July (+1d) in 1993]. Sperm-bearing sea cucumbers would have spawned sooner, if they had not been collected.

On the other hand, when they were taken one day after every full and new moon (+1d), the average ratio of fertile, pre-spawning individuals to all collected specimens was high [86.5 \pm 18.0%], and when taken three days after (+3d), the ratio was low [9.7 \pm 8.3%] (refer to Fig. 5B–C). These findings indicate that many of fertile individuals spawned three times at semilunar intervals, because they spawned out once, and further indicate that the spawning occurred during the time from the evening of +1d until the morning of +3d, because the low tide of these collection days was from one o'clock to three. The records of 1990 are fragmentary (Fig. 5A). However, during the half of July and August of 1992 and 1993, many individuals synchronously spawned three times as described above, and before and after the same season of 1990, partial and no spawnings occurred, respectively. From these observations it can be concluded that the date of the collection, 25 July 1990, corresponds with the third day of the first main spawning period (I in the Fig. 5). The identified results of 1990 were thus similar to those of 1992 and 1993.

To determine at what time of day spawning occurs, sea cucumbers were collected on +1d, and maintained in a bowl containing aired water. At the evening of the collected day, some oocytes showed migration of the germinal vesicle to the cell periphery which will be normally followed by maturation division, but neither the vesicle breakdown nor gamete release was seen until the morning of +3d.

Sexual types

The sex of *P. ooplax* was determined by examining the gonad. When specimens were collected on +1d during the main spawning season lasting from the middle of July, many of them were males with sperm, rarely with spermatocytes, and females with grown oocytes, the others being spent or sterile ones (for details, see the legend of Fig. 5). The males were further divided into two subgroups: those with smaller, growing oocytes ($\delta[\circ]$) and with no oocyte (δ), $\delta[\circ]$ having a hermaphroditic appearance under a dissecting microscope. Thus pre-spawning sea cucumbers were distinguishable into three sexual types, δ , $\delta[\circ]$, and ♀ (Fig. 3). Since the average oocyte size did not widely differ among different $\delta[\circ]$ individuals (Fig. 4), it seems that these oocytes began to synchronously grow. The percent changes in numbers of the three sexual types, the spent group, and the sterile during the main spawning season is shown in Fig.

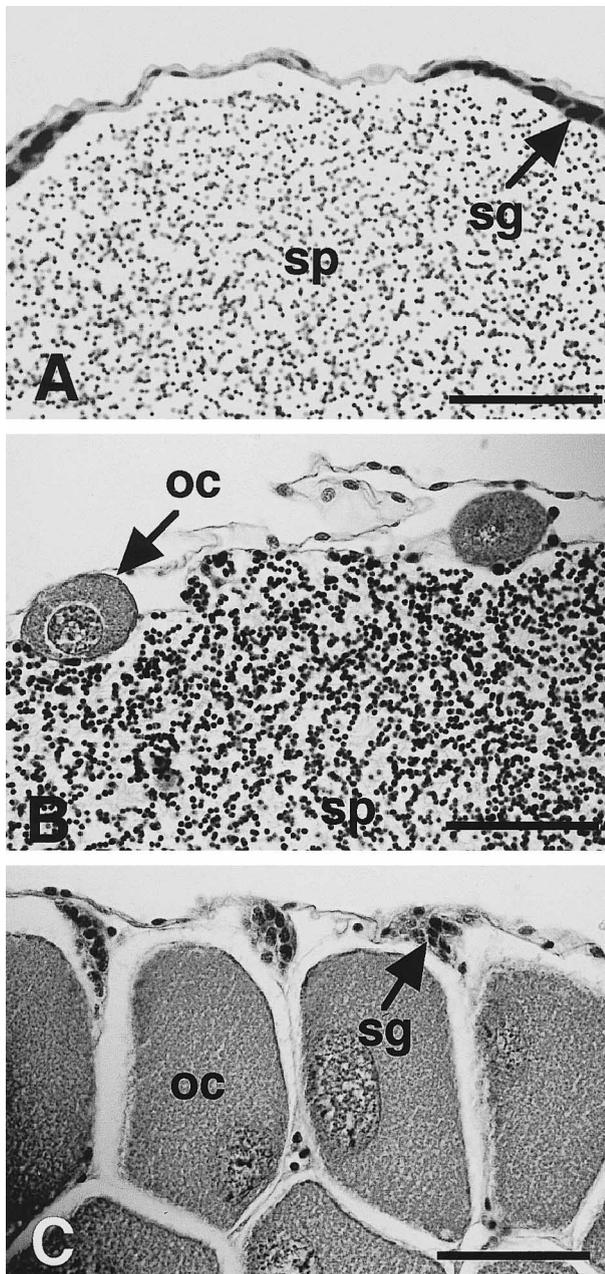


Fig. 3. Cross sections of a ♂ gonad (A), a ♂[○] gonad with small, growing oocytes (B), and a ♀ gonad (C). The gonads were fixed on +1d during the main spawning season. Note the absence of proliferating spermatogonia in the ♂[○] gonad (B). sp: sperm; sg: proliferating spermatogonia; oc: oocytes. All scale bars: 50 μm.

5. The percentages in the figures shows that a balance in individual number was preserved between sperm-producing sea cucumbers (♂ and ♂[○]) and the egg-producing (♀) at every spawning, whereas the ratio of ♂[○] to the total of ♂, ♂[○], and ♀ on +1d remarkably decreased in the third spawning periods (III) of 1992 and 1993.

Sex changes

Mature individuals spawned out their all gametes once during +1d to +3d, and ♂[○] alone bore small, growing oocytes in its gonad at each spawning time. These facts indi-

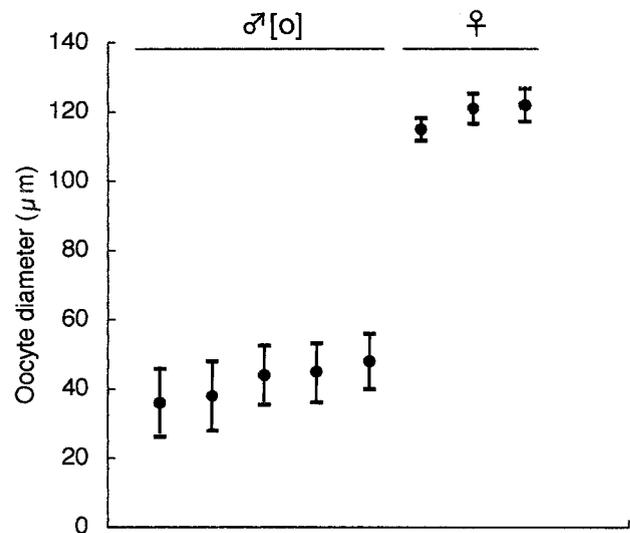


Fig. 4. Average oocyte diameters of each of five ♂[○] and three ♀ individuals (1992). Noticeably smaller diameters of oocytes of the ♂[○] indicate that these oocytes are at an early growth stage. Vertical bars: ±SD.

cate that the sex changed from male to female during the interval between the first and second spawnings. In addition, the occurrence of the inverse change from female to male during the same interval is considered based on the following reasons. First, mature ♀ individuals had proliferating spermatogonia at the time of the first spawning, and second, in both 1992 and 1993, the total percentage of fertile individuals on +1d of the second spawning periods was approximately equal to that of the first (Fig. 5B–C). Furthermore, some of the ♀ individuals at the second spawning may work as ♂ at the third again. This possibility is based on the observations that first, five of nine ♀ individuals that were collected on +1d of the second spawning period of 1992 had started spermatogonia multiplication, and second, the ♂ individuals percentage on +1d of the third period was higher than that of the previous period in spite of a decrease in the total percentage of ♂, ♂[○], and ♀ (Fig. 5B). In summary, some of *P. ooplax* changed their sex from male to female or in the reverse direction during the main spawning season. Furthermore, there is the possibility for a male to become female and again male during the one breeding season.

Gametogenesis period

Histological observations of the gonad of specimens collected on 16 July (+1d), 1992 showed that five of ten ♂ and ♀ individuals had proliferating spermatogonia, three only a few, and the remainder of two not. On the same day, ♂[○] showed an early growth stage of oocytes. These facts indicate that the period from the onset of spermatogonia proliferation to sperm release was two weeks, and that the period from initiation of oocytes growth to egg shedding was probably slightly longer than two weeks. The gametogenesis period before the main spawning season was a maximum of two months (61 days) in 1992, because an increase in the

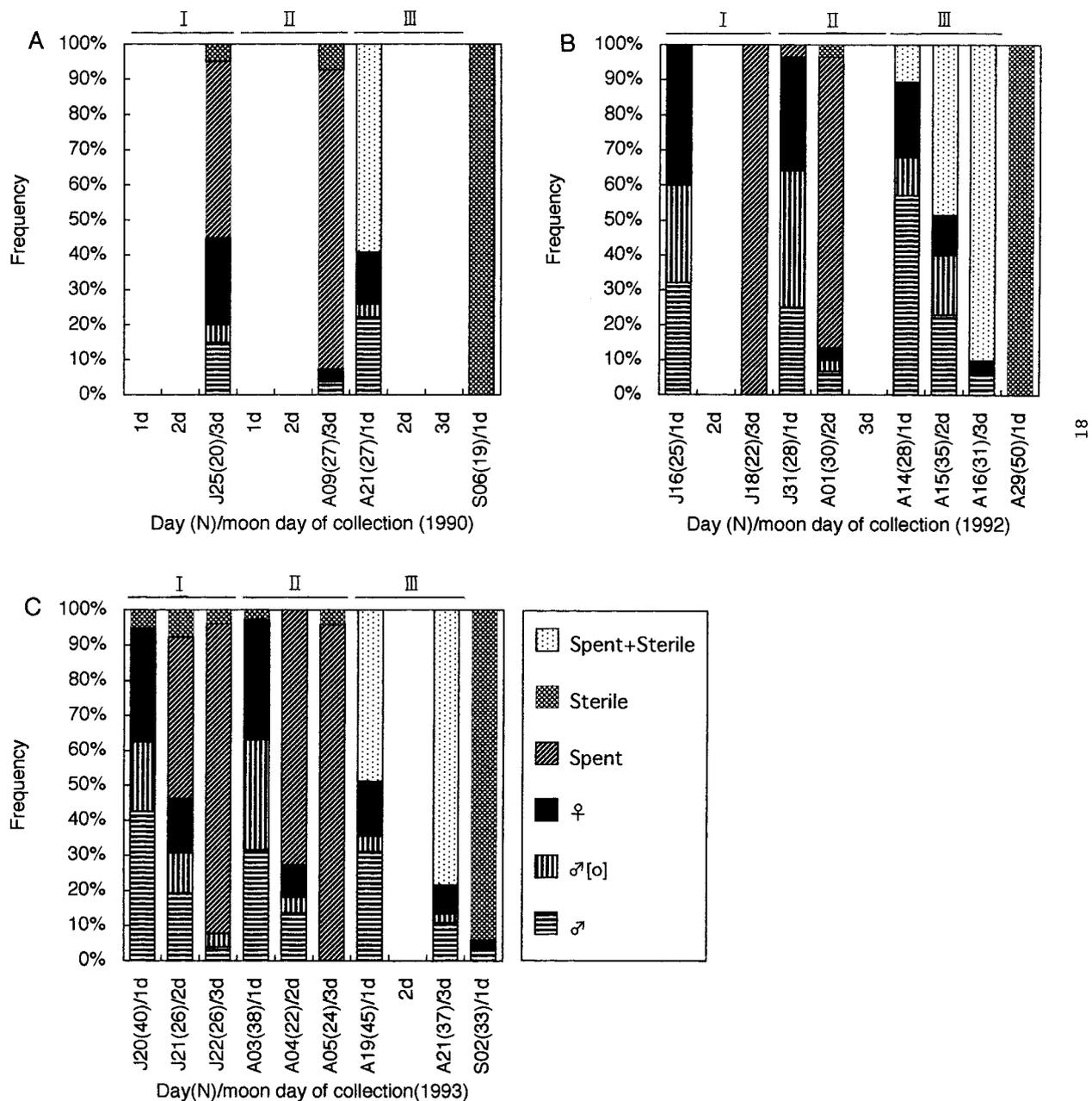


Fig. 5. Change in percentage frequencies of three sexual types, spent, and sterile groups in specimens collected on +1d to +3d during the main breeding season of 1990 (A), 1992 (B), and 1993 (C). In the first and second spawning periods (I and II in Figures), the distinction between the spent gonad and the sterile one was easy: the former was empty and as large as the prespawning gonads, and the latter was very minute, and most of it were difficult to recognize it at first glance. However, in some gonads of the third period (III), an accurate distinction between the spent gonad and the sterile was not possible due to their significant shrinkage. In specimens collected on +1d after a further two weeks, the gonads without developed reproductive cells were classified as sterile because nearly all of those gonads were of a very minute size. Numbers in parenthesis: total number of examined individuals.

gonad index began between the two collection days, 26 May and 28 June, and many individuals spawned first on 25 July.

DISCUSSION

The present paper focuses on the spawning associated with the moon phases and sex change occurring during the interval between the spawnings. The spawning time of day in

the field and in the laboratory were not ascertained. However, it is probable that the time was the two evenings of +1d and +2d, not the two mornings of +2d and +3d, since the occurrence of germinal vesicle migration was in the evening, and in summer the full tide is higher in the evening than in the morning, and the higher tidemark is more favorable for wide dispersal of the *zygotes* of intertidal animals. Indeed, the intertidal apodid *P. rufescens* spawns in the evening, presum-

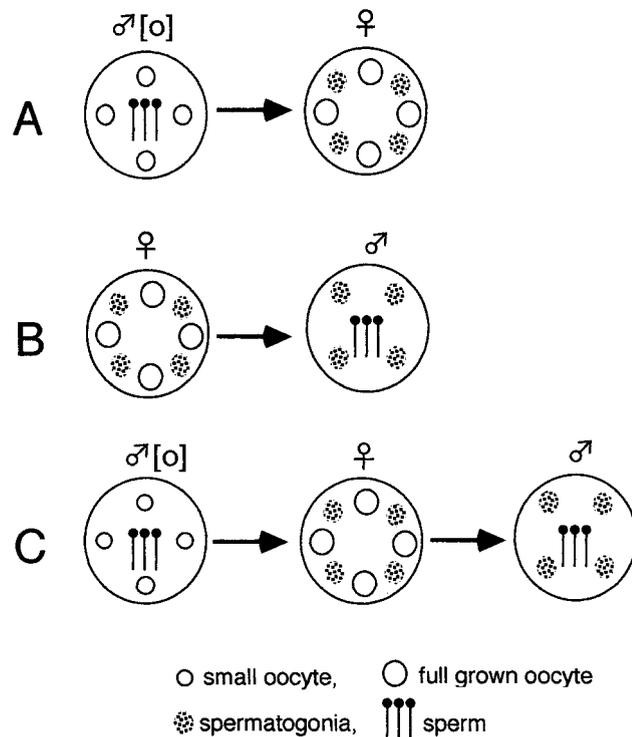


Fig. 6. Schematic illustration of reproductive cells in the gonadal tubule (the largest, outer circle) of ♂, ♂[o], and ♀ individuals, and the directions of sex change occurring at the interval between spawnings. (A) Change from ♂[o] to ♀. (B) Reversely from ♀ to ♂. (C) A possible case from ♂[o] to ♀ and then to ♂ (secondary male). For details, refer to the text.

ably after the ebb tide began (Kubota and Tomari, 1998).

The spawning behavior of holothurians has been observed by many investigators (summarized by Smiley *et al.*, 1991). Among these animals, *P. ooplax* in this study resembles the apodid *P. rufescens* (Kubota and Tomari, 1998) in the mode of spawning as follows. In both species, the spawning associated with certain moon phases began around the second half of July and lasted until the end of the breeding season. These spawnings probably occurred on days one and two after every full and new moon in *P. ooplax*, and on two and one before it in *P. rufescens*. *P. ooplax* spawned three times at semilunar intervals, while *P. rufescens* averaged five times. Furthermore, these predictable spawnings were observed in most or many of the fertile individuals in the population studied, and each individual released all the gametes at one time. Babcock *et al.* (1992) observed on the Great Barrier Reef that holothurian species such as *Bohadschia argus*, *Euapta godeffroyi*, *Stichopus chloronotus*, and *S. variegatus* spawn during one to three days of certain lunar phases. However, in most cases the occurrence of spawning was limited to a portion of the population.

Hermaphroditism has been reported in more than ten species of holothurians including eight apodids [*Leptoplax buskiee*, *Leptosynapta clarki*, *L. inhaerens*, *L. minuta*, *L. tenuis*, *Rhabdomolgus ruber*, *Synapta vivipara*, and *Polycheira rufescens* (Green, 1978; Smiley *et al.*, 1991; Sewell, 1994;

Chao *et al.*, 1995; Kubota and Tomari, 1998)]. *P. ooplax* was also hermaphroditic. Matured individuals collected on +1d during the main spawning season were divided into three sexual types, males, males with small oocytes of an early growth stage, and females. The male with the larger but unmaturing oocytes of a late stage was regularly found in *P. rufescens*, but not in *P. ooplax*. The growth-maturation period of oocytes during the main season was probably a little longer than two weeks in *P. ooplax*, being shorter than about five weeks for *P. rufescens*. The period from the initiation of spermatogonia to sperm release was two and three weeks in *P. ooplax* and *P. rufescens*, respectively. Similarly, the gametogenesis period before the main spawning season known from the change in the gonad index was a maximum of two months in *P. ooplax*, while being shorter than three months in *P. rufescens*. In the latter species, the rising of the gonad index coincides in time with the histological onset of gametogenesis in this year.

As the sex changer in echinoderms, 15 species have been described (Policansky, 1982; Sewell, 1994; Kubota and Tomari, 1998). Among holothurians, a sex change was revealed in two apodids, *Leptosynapta clarki* (Sewell, 1994; Sewell and Chia, 1994) and *P. rufescens* (Kubota and Tomari, 1998), and suggested in *Holothuria atra* (Harriott, 1982) and two apodids *Leptosynapta inherens* (Runström, 1927) and *Lepidoplax media* (Gotto and Gotto, 1972). These echinodermal sex changers are all protandric (Sewell, 1994) except for *P. rufescens*; a single individual changes from the young, small male to the old, larger female during its lifetime. The mode of sex change for *P. ooplax* is the same as that of *P. rufescens* such that the change reciprocally occurs from male to female or in reverse in two-week intervals between spawnings.

In addition, there is the possibility that some *P. ooplax* individuals underwent male, then female, and returned to male. In this case, males with oocytes at the first spawning functioned as female with proliferating spermatogonia at the second spawning, and participated as the secondary male at the third. The change from female to the secondary male was ascertained in tagged individuals of *P. rufescens*, and histologically suggested in the protandric species *Leptosynapta clarki* by Sewell (1994). Although the proposal of Ghiselin (1969) that the evolution to protandry is based on size advantage has been widely accepted, what the selective significance there is to such reciprocal and possibly alternate sex changes in *P. ooplax* is not clear.

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