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Reproductive Periodicity of a Tropical Dendrochirote Holothurian, *Phyrella fragilis* (Echinodermata: Holothuroidea), in Taiwan[1]

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Shyh-Min Chao, Chang-Po Chen and Paul S. Alexander (1993) Reproductive periodicity of a tropical dendrochirote holothurian, *Phyrella fragilis* (Echinodermata: Holothuroidea), in Taiwan. *Bull. Inst. Zool., Academia Sinica* **32**(2): 111-119. The reproductive periodicity of the dendrochirote holothurian *Phyrella fragilis* (Ohshima) was studied from April, 1990 to July, 1991 on the intertidal flats of Nanwan (21°57'N, 120°45'E) in southern Taiwan. Both gonad index and histological examinations show that *P. fragilis* has a short, discrete breeding period from April to May. After a post-spawning period in June and July, gametogenesis resumes in August and lasts until the following March. The large mature eggs of *P. fragilis* measuring 350 ± 50 μm in diameter, suggests lecithotrophic larval development. The shallow-water dendrochirote holothurians of both Taiwan and temperate areas tend to display short breeding periods during warm months.

Key words: Reproduction, Sea cucumber, *Phyrella fragilis*.

The dendrochirote holothurians of shallow temperate waters generally display a discrete and short breeding period (Hyman 1955, Boolootian 1966, Costelloe 1985, McEuen 1987, McEuen and Chia 1991). However, it is not known if this generalization fits tropical dendrochirotids. There have been few studies conducted on the reproductive periodicities of tropical dendrochirote holothurians, possibly due to a shortage of materials required for gonad index calculations. The dendrochirote holothurians of southern Taiwan are tropical, thus providing an opportunity to compare their reproductive periodicities with those of species that live in higher latitudes.

Only two species of dendrochirote holothurians occur in the shallow waters off the Taiwan coast (Chao and Chang 1989). One of them, *Phyrella fragilis*, is distributed throughout the waters of the East Indies, China, and southern Japan (Clark and Rowe 1971); they commonly occur in the mid-

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intertidal flats of southern Taiwan. Although the body surface of *P. fragilis* is densely covered by tubular feet, it does not move freely; instead, it burrows in sand in a "U" shape, and passively traps food by extending its dendriform tentacles into the water column. It is worth mentioning that this species is considered a great delicacy, especially in Asia (Chang and Liao 1964).

The goal of the present study is to describe the reproductive periodicity of *P. fragilis*. Correlations between reproductive periodicity and both food availability and ambient seawater temperature are addressed. A comparison of reproductive periodicities in shallow water dendrochirotids found in tropical southern Taiwan and temperate areas is also discussed.

MATERIALS AND METHODS

Specimen collection

During the lowest spring tides of April, 1990 through July, 1991, 12-15 adult (>30g wet body weight) *Phyrella fragilis* (Ohshima) specimens were collected monthly from the intertidal flats at Nanwan (21°57'N, 120°45'E) in southern Taiwan. Individuals were relaxed in aqueous magnesium sulfate (about 80 g $MgSO_4 \cdot 7H_2O$ per one liter seawater) for 3 hours, then injected with 10 ml of 20% formalin.

Determination of sex and calculation of gonad index

Individuals were dissected, and their gonads, guts (including food) and body walls were drained and weighed to 0.01 g. Individuals were sexed by microscopic examination of gonads. Gonad indexes and gut-content indexes were calculated according to the following formulas:

$$\text{Gonad index} = \frac{\text{gonad weight}}{\text{body wall weight}}$$

$$\text{Gut-content index} = \frac{\text{gut weight}}{\text{body wall weight}}$$

Histology of gonads

Portions of gonads from each individual were fixed in Bouin's fluid for one day, then dehydrated in graded alcohols, cleared in xylene, and embedded in paraffin wax. Sections (5 μm thick) were cut with a rotary microtome, then stained with Mayer's hematoxylin and eosin. Gonad development was divided into five stages: recovery, growth, mature, shedding, and post-spawning; these are similar to those of *Stichopus japonicus* Selenka (Tanaka 1958) and *Aslia lefevrei* (Barrois) (Costelloe 1985).

Seawater temperatures

Seawater temperatures were measured monthly on the bottoms of three permanent tidepools of about 0.5 m in depth at noon and midnight at the study site; seawater temperatures were calculated ($\bar{\chi} \pm$ SD) and plotted.

RESULTS

Morphology of sexes and gonad description

Phyrella fragilis is gonochoric. Mature testes and ovaries consist of a single tuft of tubules (25 ± 5 mm in length and 1 ± 0.2 mm in diameter, $N = 15$) on the dorsal mesentery in the central visceral coelom. Mature gonads are creamish-white in males and green in females. Among 229 individuals collected, 126 individuals were sexable; 66 (52%) were female and 60 (48%) male. The χ^2 test suggests an even male-to-female ratio ($\chi^2 = 0.29$, $df = 1$, $p < 0.05$) in this

population.

Oogenesis

The recovery stage is marked by a thickening of the coelomic epithelium of the gonad tubules (80 ± 20 μm). The lumen is very small, and oocytes are not present. Distinct oogonia cannot be identified at this stage (Fig. 1A).

The active production of oocytes characterizes the growth stage. Tubules grow to 1 mm in diameter without much reduction in the thickness of the coelomic epithelium (70 ± 20 μm). Developing oocytes (75 ± 25 μm in diameter) lie in the germinal layer and are surrounded by small accessory cells as described for *Ypsilothuria talismani* Perrier (Tyler and Gage 1983) and *Aslia lefevrei* (Costelloe 1985) (Fig. 1B).

In the mature stage there is a great reduction in the thickness of the coelomic epithelium (35 ± 15 μm) as oocytes continue to grow until they approach shedding size (350 ± 50 μm in diameter). In the late stages of development, each oocyte is surrounded by a follicular membrane. At this stage, many oocytes degrade to form numerous enclosed spherule cells (Fig. 1C).

The shedding stage is characterized by a very thin gonadal wall (15 ± 5 μm) and large lumen filled with large mature eggs (350 ± 50 μm in diameter). In this stage, many ova continue to degrade to form enclosed spherule cells. Also at this stage, eggs lose their follicular membranes and are prepared for spawning. Gonad walls are transparent, and eggs can be readily identified by the naked eye (Fig. 1D, E).

The main characteristic of the post-spawning stage is the presence of sparsely distributed-residual ova at various stages of resorption. Except for some primary oocytes, these residual ova are phagocytosed, thus forming spherule cells (Fig. 1E).

Spermatogenesis

As in oogenesis, the recovery stage is marked by a thickening of the coelomic epithelium (120 ± 30 μm). The germinal lumen remains very small as clusters of numerous spermatogonia begin to grow. At this stage, no spermatids or spermatozoa can be found in the lumen (Fig. 2A).

The growth stage is characterized by the active production of spermatocytes which line the inner wall of the germinal layer. The coelomic epithelium reduces in thickness to 60 ± 10 μm, allowing a larger lumen to appear (Fig. 2B). Spermatids and spermatozoa may be present, often detached and filling the lumen. Unlike *Aslia lefevrei* (Costelloe 1985), which has a distinct spermatid layer, there is no border between the spermatids and spermatozoa of *P. fragilis*; they usually occur together. Mature spermatozoa are usually produced and concentrated in the central lumen (Fig. 2C).

The mature stage is characterized by a further reduction in the thickness of the coelomic epithelium (25 ± 5 μm) and by an increase in lumen space. The tubules increase to 1 mm diameter as the spermatocyte layer thickness is reduced. As in the growth stage, there is no distinct border between the spermatid layer and spermatozoa. At this stage, the lumen is densely filled with spermatozoa (Fig. 2D).

Compared to the mature stage, during the shedding stage the thickness of the coelomic epithelium does not decrease (25 ± 5 μm). Spermatocytes almost disappear completely. Following spawning, the densely-packed spermatozoa separate from the germinal epithelium, resulting in the existence of a space between the spermatozoa and the germinal layer (Fig. 2E).

As with female *P. fragilis*, spermatozoa is not completely shed in a single reproductive season. During the post-spawning stage, most residual sperm often remain

in the lumen to be reabsorbed. However, unlike the resorption of residual ova, no spherule cells or phagocytes were identified (Fig. 2F) in this study. In several tubules, residual spermatocytes were not reabsorbed.

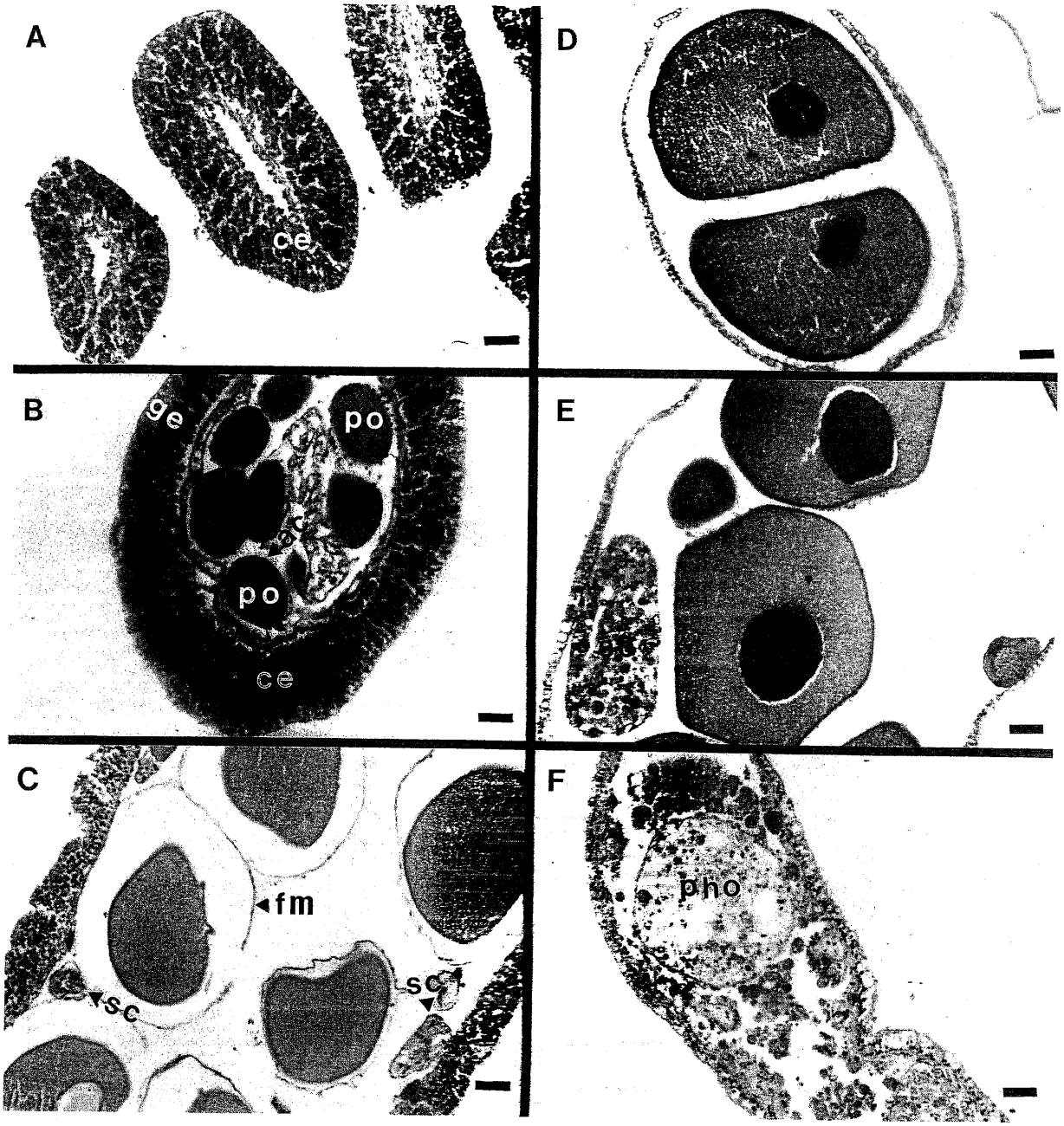

Fig. 1. Female gametogenic stages of *Phyrella fragilis*: (A) recovery stage in September, (B) growth stage in December, (C) mature stage in March, (D and E) spawning stage in May and (F) post-spawning stage in July. ac: accessory cells; ce: coelomic epithelium; fm: follicular membrane; ge: germinal epithelium; pho: phagocytosed ovum; po: primary oocyte; sc: spherule cells. Scale = 50 μm.

Gonad index and maturity

Gonad index peaked at 0.32 in April, 1990, then dropped sharply to 0.08 in June. It increased gradually to 0.21 in November, then dropped to 0.13 in January, 1991, followed by rapid growth to 0.22 in February. The index peaked again to 0.22-0.24 from

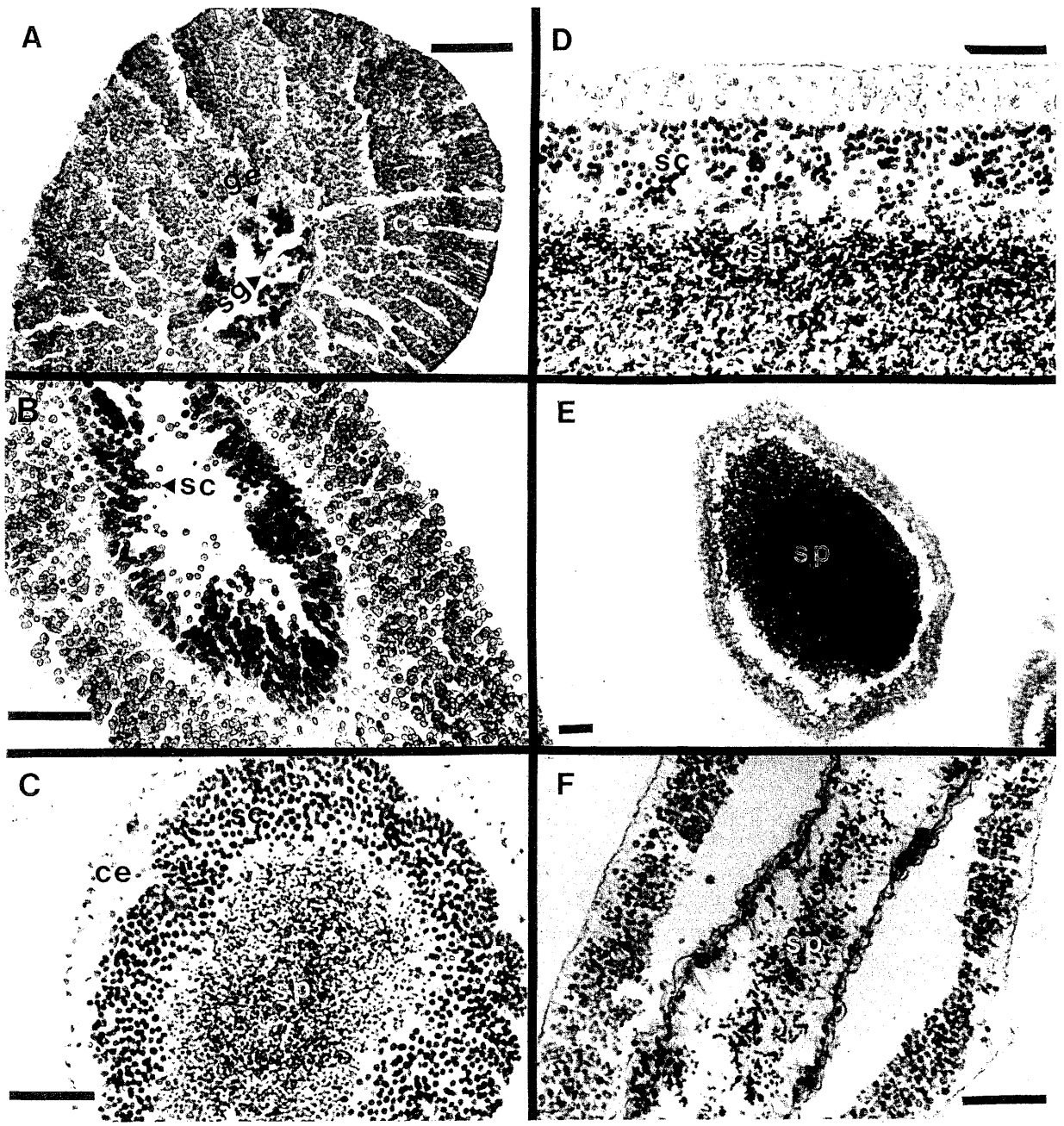

Fig. 2. Male gametogenic stages of *Phyrella fragilis*: (A) recovery stage in September, (B and C) growth stage in December, (D) mature stage in February, (E) spawning stage in May and (F) post-spawning stage in July. ce: coelomic epithelium; ge: germinal epithelium; sc: spermatocytes; sg: spermatogonia; sp: spermatozoa. Scale = 50 μm.

February through April, 1991, decreased rapidly to 0.09 in June, then maintained this low value through July. The gonad index calculated for our study showed an approximate four-fold increase in gonad-to-body wall weight over time (Fig. 3A).

A maturity chart of 229 individuals revealed that gonads are shed mainly in April and May, recover in August and September, grow from October to January, and mature in February and March (Fig. 4).

Gut-content index

Gut-content data for eleven months are available. The gut-content index was lowest (0.20) in September, 1990, then increased sharply and peaked in November (0.65). It dropped sharply (0.27) in January, 1991, then increased (to 0.44) in February; it decreased gradually (to 0.21) in June (Fig. 3B).

Fig. 5 shows the simple linear regression between gonad index and gut-content

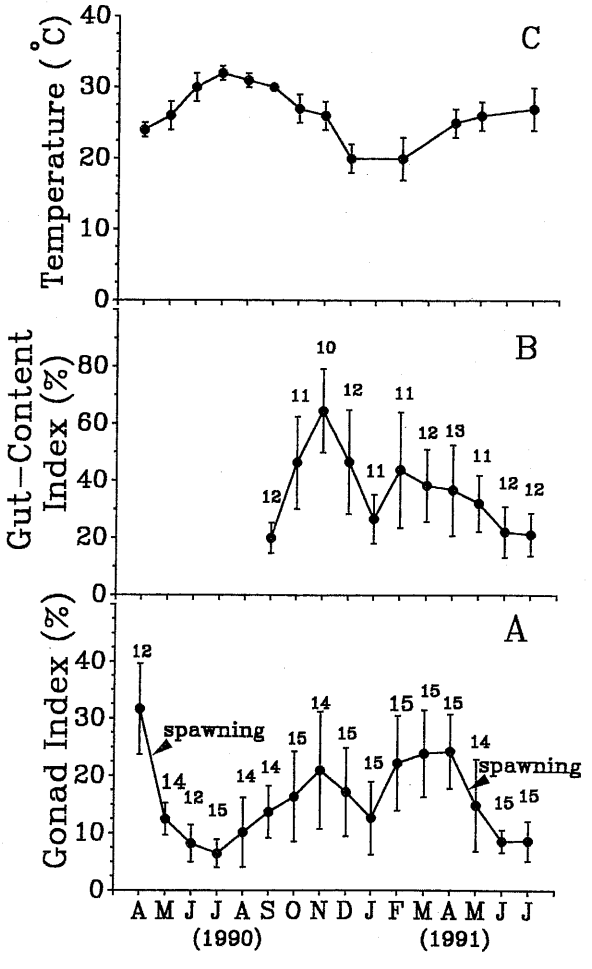

Fig. 3. Gonad index (A) and gut-content index (B) of *Phyrella fragilis* and seawater temperatures (C). Vertical bar indicates ± SD. Numbers above vertical bars indicate sample size.

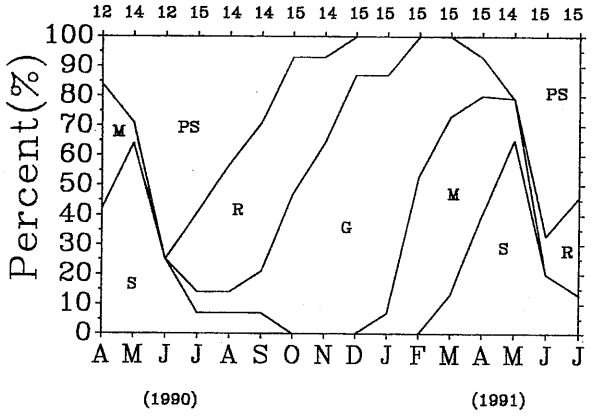

Fig. 4. Maturity chart for *Phyrella fragilis*. Numbers on top indicate sample size. G = growth stage, M = mature stage, PS = post- spawning stage, R = recovery stage, S = shedding stage.

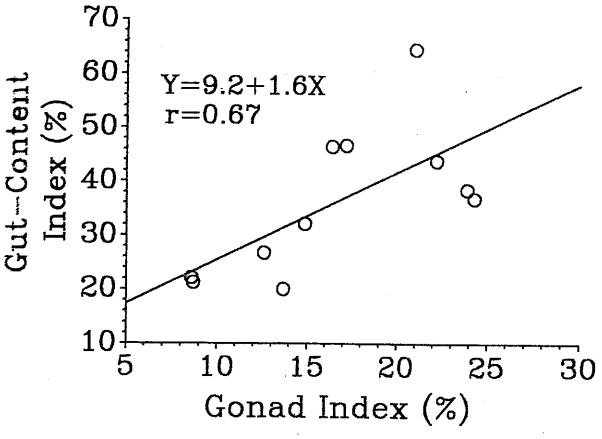

Fig. 5. One-way linear regression between gonad index and gut- content index of *Phyrella fragilis*.

index, indicating a significant positive correlation between these two indexes ($r = 0.67$, $F = 7.26$, $p < 0.05$).

Seawater temperature

Seawater temperature in April, 1990 was $24 \pm 1°C$, increasing and peaking in July at $32 \pm 1°C$. From November it decreased sharply to a low of $20 \pm 3°C$ in February, 1991, after which it began to increase again (Fig. 3C).

DISCUSSION

Although *Phyrella fragilis* is gonochoric, sex can only be determined by color or the histological preparation of gonads. There is no correlation between sex and any external characteristic or behavior. No evidence was found for either simultaneous or sequential hermaphroditism.

Dendrochirote holothurians tend to produce large ova (250-450 μm in diameter) which usually undergo lecithotrophic development (Costelloe 1985, McEuen 1987 1988, McEuen and Chia 1991). The ova of *P. fragilis*, measuring 350 ± 50 μm in diameter, also suggest the lecithotrophic development of larvae.

Costelloe (1985) reported that in *Aslia lefevrei* the decrease in gonad index after initial recovery may be explained by nutrient assimilation. For *Phyrella fragilis*, there is no decrease in gonad index during the recovery stage, but the thickness of both the germinal layer and coelomic epithelium are greatly reduced as gametogenesis progresses. During the late growth and mature stages, the gonad index of *P. fragilis* decreases sharply; histological examination reveals that this decrease is not due to spawning, but that numerous oocytes are phagocytosed during this period. As this decrease is too large to be explained simply by phagocytosis, it is possible that it is due to a variation of samples, indicating the need for further study.

Aslia lefevrei displays new sex-cell (primary oocyte) growth for the coming year prior to the release of mature gametes (Costelloe 1985); he suggested that this indicates no change in the sexuality of *A. lefevrei*. Similar to *A. lefevrei*, during the post-spawning and recovery stages the primary oocytes of *P. fragilis* are not completely phagocytosed.

The gut-content index increase from August to November, synchronized with an increasing gonad index during the recovery and growth stages, suggests that more food is required for gonadal growth. Histological examination shows a nutrient buildup in the gonad wall of *P. fragilis* during this period. During the mature and spawning stages, the gut-content index decreases, indicating that less food is required. Histological examination also reveals that the gonad wall reduces in thickness during this period; this suggests that *P. fragilis* undergoes reproductive buildup during late summer and autumn, and that nutrients are assimilated by gametes during the cooler months. This is similar to *A. lefevrei* (Costelloe 1985), which exhibits its greatest nutrient buildup during the warmer months, thereafter relying on those food reserves for general metabolism and gametogenic activity during winter.

For holothurians, reported extrinsic factors controlling their reproductive cycles include temperature (Tanaka 1958, Bakus 1973, Conand 1981, Costelloe 1985, Sewell and Bergquist 1990), photoperiod (Conand 1982, Costelloe 1985), salinity (Krishnaswamy and Krishnan 1967), and current and crowding (Engstrom 1980). Many temperate holothurians have been reported to spawn in spring, when water temperature increases (Choe 1963, Cameron and Fankboner 1985, Costelloe 1985, McEuen 1987 1988, McEuen and Chia 1991). Sewell and Bergquist (1990)

reported that the delay of spawning in *Stichopus mollis* (Hutton) for two to three months in 1987 was due to abnormally low seawater temperatures. Bakus (1973) reported that changes in water temperature, particularly increases, induce breeding in some temperate holothurians. In both 1990 and 1991 *Phyrella fragilis* spawned in April and May, when seawater temperature increased; this is a breeding pattern similar to that of temperate holothurians.

Dendrochirote holothurians in higher latitudes tend to spawn during warm months (Costelloe 1985, McEuen 1987 1988, McEuen and Chia 1991). In southern Taiwan, *P. fragilis* spawns in early spring at the beginning of the warm season. The other dendrochirote species found in southern Taiwan, *Afrocucumis africana*, also spawns in early spring (pers. obser.). It appears that the shallow-water dendrochirotids found in both Taiwan and temperate zone waters tend to spawn during the warm months.

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台灣產熱帶性海參：脆沙雞子參生殖週期之研究

趙 世 民　陳 章 波　歐 保 羅

自 1990 年 4 月起至 1991 年 7 月止，每月在台灣南部之南灣地區，採集生活於潮間帶之樹手目海參：脆沙雞子參，以進行生殖週期之研究。生殖腺之指數及組織切片顯示：脆沙雞子參於每年之 4、5 月排卵。生殖腺由 8 月開始恢復發育，至次年 3 月停止產生配子。由其所產生之大型卵（卵徑爲 350±50 μm）推測，其幼蟲發育屬於卵營養型。台灣及溫帶地區沿岸之樹手目海參有一共同生殖趨勢：生殖季短且集中於溫暖月份。

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Progesterone-like Substance in the Ovaries, Hepatopancreases, and Hemolymph of Female *Uca arcuata*

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Jin-Taur Shih and Yuh-Ming Wang (1993) Progesterone-like substance in the ovaries, hepatopancreases, and hemolymph of female *Uca arcuata*. *Bull. Inst. Zool., Academia Sinica* **32**(2): 120-126. Our purpose was to find whether or not fiddler crab (*Uca arcuata*) tissues contained a progesterone-like substance. Ethanol extracts were prepared from the ovaries, hepatopancreases, and hemolymph of female *U. arcuata*. Using a chromatograph with a RP-C18 colum, a progesterone-like substance was identified in all samples of extracted steroid residues according to a retention time similar to a progesterone standard. All samples showed a positive reaction to progesterone via radioimmunoaasay.

Key words: *Uca arcuata*, Ovary, Hepatopancreas, Hemolymph, Progesterone-like substance.

A sex steroid-like substance in crustaceans was first found in the ovaries and eggs of the American lobster (*Homarus americanus*) (Donahue 1940, 1948). Sasser and Singhas (1988) used column chromatography and radioimmunoassay (RIA) to analyze the hemolymph of *Callinectes sapidus* and penaeid shrimp; an estradiol-like substance was found in both species. Recently a progesterone-like substance as identified via high pressure liquid chromatography (HPLC) was found in the body extract of female *Uca arcuata* (Shih *et al.* 1990). Shih (1992) also reported finding both progesterone-like and estradiol-like substances in the hemolymph of *Uca arcuata*, and that these sex steroid-like substances reached peak levels two to three months before breeding.

In an *in vitro* study, Sandor (1981) reported that ovarian tissue in *Portunus trituberculatus* converted progesterone to 11-deoxycorticosterone. Enzymes involved in the conversion of progesterone to hydroxyprogesterone, testosterone and deoxycorticosterone have been isolated in the ovaries of *Portunus trituberculatus* (Teshima and Kawazawa 1971). In addition, ecdysteroids were detectable by RIA in the ovaries of *Carcinus maenas* (Lachaise and Hoffmann 1977). Ecdysteroids have also been detected in the oocytes, follicle cells and hemolymph of *Orchestia gammarellus* (Zerbib 1976, Rateua and Zerbib 1978, Blanchet *et al.* 1979).

There is still a lack of either qualitative or quantitative evidence showing the existence of a crustacean (crab) containing sex steroids in its ovaries, hepatopancreases, adipose tissues and/or hemolymph. Furthermore, the location of sex steroid biosynthesis in crustaceans is still unknown. However, before we can find this location, it is im-