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Sexual reproduction in a fissiparous holothurian species, *Holothuria leucospilota* Clark 1920 (Echinodermata: Holothuroidea)

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Abstract

Holothuria leucospilota Clark 1920 inhabiting tropical Darwin waters primarily undergo asexual reproduction by fission throughout the year (Purwati 2001). However, there is also evidence of sexual reproduction. Monthly sampling from August 1998 to January 2000 revealed that the gonadal tubules within each individual of *H. leucospilota* grew simultaneously. Complete spawning, where the oocytes throughout the gonad have equal opportunity to be released in one spawning event, could therefore be expected. Post-spawning tubules were absorbed, resulting in the disappearance of gonads between reproductive cycles. The development of gonadal tubules in this holothurian does not conform to the "tubules recruitment model" proposed by Smiley (1988), as reassessed by Sewell et al. (1997).

A seasonal reproductive cycle with a restricted spawning period was recognised in the population studied. The resting stage that occurred simultaneously amongst individuals in the population made it possible to estimate gametogenesis, which may take less than a year. It is likely that gamete release occured in the period between new moon and full moon of April, at the end of the wet season in Darwin. The continuous flooding of the reefs during this period is thought to be effective for fertilisation.

Introduction

Variations occur in holothurian gonad structures and development (Conand 1981; Harriott 1985; Tuwo and Conand 1992; Hamel et al. 1993; Conand et al. 1997). In a holothurian population where the gonads develop simultaneously and spent tubules are absorbed after the reproductive season, the gonads may not be visible for a certain period. However, intraspecific variation can occur, such as in *Stichopus mollis* of New Zealand, where the population from the east coast of the North Island absorbs the after-spawned tubules and the gonadal basis, whereas the population in the South Island maintains its spent tubules (Sewell 1992). Intraspecific variation in number of gonad tufts is also possible. One example is dendrochirote *Cucumaria frondosa*, in which geographical and latitudinal factors are the suggested influencing factors (Sewell 1992; Hamel and Mercier 1996).

In view of these variations occurring amongst holothurian populations, it was of interest to study the sexual reproduction of *Holothuria leucospilota* from Darwin harbours, northern Australia. The investigation aimed to elucidate the types of gonadal

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tubule recruitment, and synchrony and seasonality of gonad development over the population. *H. leucospilota* has been observed to reproduce asexually by fission throughout the year (Purwati 2001; Conand et al. 1997) and this fission may influence the sexual reproductive activities.

Materials and methods

Individuals of *H. leucospilota* were collected from East Point reef, Darwin, northern Australia (12°24.20'S and 130°49.20'E). The population occupied an area of approximately 500 x 700 m with a relatively low density (0.077–0.29 individuals m^{-2}) and was composed of small animals, mostly less than 350 g in fresh body weight.

Tubule fractions of 170 gonads in total, sampled from individuals weighing 200 g or more in fresh body weight, were examined. This size was determined after a preliminary study revealed that individuals weighing at least 200 g in fresh condition carried gonads. Collections were conducted once every month from August 1998 to January 2000, except in April 1999 when sampling was carried out twice (15 and 29 April). Sample size ranged from 5 to 18 individuals per month, the inconsistency being due to the conservation policy practised on the study sites.

A small incision was made on the antero-dorsal integument of each individual to remove fractions of gonadal tubules from the body cavities. This was made on the site and the dissected bodies were returned to the reef. Chao et al. 1994 utilised this method and reported that the scar on the dorsal part of *Holothuria atra* caused by incision disappeared within six months. During our study, two individuals with scar at the point of incision were observed on the reef of East Point approximately 8–11 months after the first sampling.

Gonads collected were classified into four stages: early, growing, fecund and post-spawning stage, based on morphology and histology of the gonads. Tubule fractions were prepared for microscope slides by applying 10 per cent buffered formaldehyde fixation, paraffin procedures and hematoxylen-eosin staining. A camera lucida was set up on a compound microscope to enable diagrams of the ovarian histology to be drawn. Data on the environmental conditions provided by the Bureau of Meteorology Darwin were used to identify possible clues to the factors leading to spawning.

Results

Characteristics of the gonadal tubules

Gonadal tubules of *H. leucospilota* hang freely in the body cavity from a transparent saddle-like gonadal basis located at the side of the anterior part of the intestine. A simple gonoduct emerges from the gonadal basis and ends at the gonopore, approximately 2–3 cm from the oral end. Tubules were observed to protrude from the gonadal basis in two rows. Each tubule was straight and bifurcated twice or three times, rarely exceeding four times. The dimension, colour and number of the tubules were correlated with the stages of development (Fig. 1).

Male tubules were always creamy white in colour. Female tubules were more transparent, with the interior having a granulated appearance. The female tubules became reddish-orange with the development of fecund ovaries. After spawning, the tubules deteriorated and turned brown; unspawned oocytes were likely to be reabsorbed. Of the individuals sectioned, 59 were males, 64 were females and 47 either carried unidentified gonads or had no visible gonads. The number of males was calculated to be similar to the number of females, in which *x* observed (0.2030) was smaller than *x* expected (3.841) (d.f. +1; p = 0.05).

Table 1 gives the dimensions of the ovarian tubules and oocytes at various stages. The smallest ovaries were in the early development stage. They consisted of 7 transparent tubules, less than 8 mm long and 0.01 g fresh weight. The largest ovary in fecund condition consisted of more than 14 large orange tubules weighing 81.92 g fresh weight.

Table 1. **Size of ovarian tubules and oocytes at different stages of development**

Ovarian stage of development	Ovarian tubules		Oocyte
	Length	Diameter	Diameter
Early developing	≤70 mm	≤0.9 mm	5–60 mm
Growing	22–25 cm	<2.5 mm, but may reach 4 mm	5–110 mm
Fecund	20–30 cm, may reach 40–45 cm	4–5 mm	120–140 mm
Post-spawning	Varied	Shrinking	Large oocytes remained

Advanced ovaries were heavier as a consequence of increased gamete number and volume. Tubules protruding from the anterior part of the basis were frequently shorter but diameter, coloration and internal appearance were similar to the others.

All tubules within each individual gonad were always at the same stage of maturity. Furthermore, gonads collected in one sampling event tended to be in a similar state of maturity, indicating synchronous progression throughout the population (Fig. 2). A seasonal pattern can therefore be expected.

Stage of development

Figure 2 illustrates the pattern of development observed between August 1998 and January 2000. The early stage of development was mainly found in October–November 1998 and again in August–September 1999. Growing gonads were gathered among samples collected in December 1998–January 1999 and December 1999. From January to early April 1999 and in January 2000, collected gonads were mostly in fecund condition. In the last week of April through June 1999, post-spawning

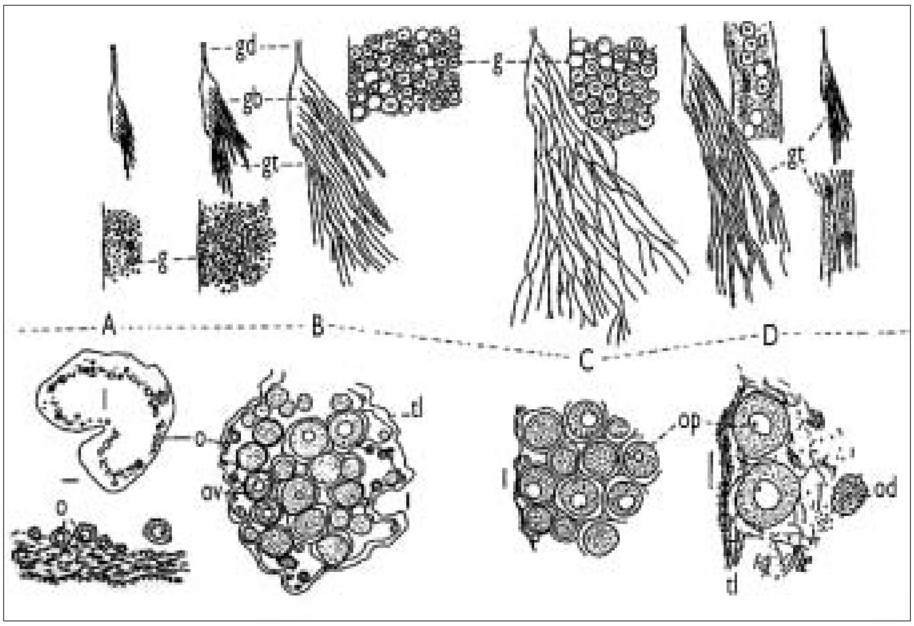

Figure 1.

Ovarian tubules of *H. leucospilota* under dissecting (above line) and compound microscope (below line). A: early stage; B: growing stage; C: fecund; D: post spawning. gb: gonadal base; gd: gonoduct; gt: gonadal tubules; l: lumen; o: pre-vitellogenic oocytes; od: degenerated oocytes; op: postvitellogenic oocyte; ov: vitellogenic oocyte; t: tubule; tl: tubule lining. Scale bars in A, B, C, and D: 20, 40, 50 and 50 µm

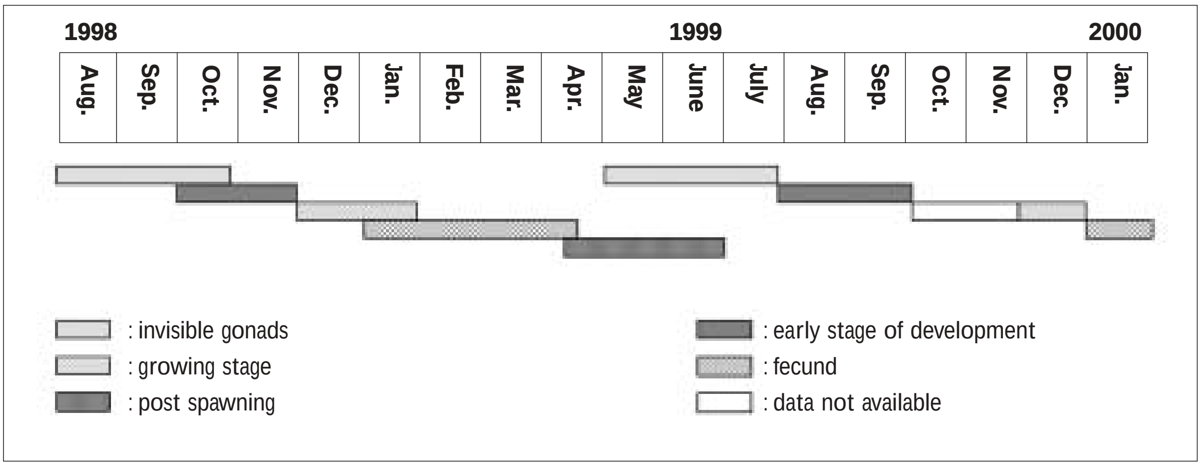

Figure 2. **Condition of collected gonads**

gonads were common and several of them were being absorbed. A similar condition was detected in gonadal sections in August, September and October 1998.

These observations indicate that oocyte release occurred in late April. This was between new and full moon, coinciding with almost continuous flooding of the reefs. April also marked the end of the wet season and the commencement of the dry season, when mean daily bright sunshine increases steeply and precipitation gradually decreases to a minimum (BOM data, in Purwati 2001).

Discussion

Development of gonadal tubules

Individuals of *H. leucospilota* at East Point as well as those from tropical southern Taiwan (Chao et al. 1995) and Vietnam (Nguyen and Britayev 1992), and subtropical Heron reef (Franklin 1980), Hong Kong (OngChe 1990) and La Réunion (Conand et al. 1997), possess a single tuft of gonadal tubules. Intraspecific variations such as those reported in dendrochirote *Cucumaria frondosa* (Hamel and Mercier 1996) due to influences of geographic position and latitudinal difference are unlikely to occur for *H. leucospilota*.

Gonadal tubules of each individual studied developed simultaneously and became fecund at the same period, similar to the same species at subtropical Heron Island (Franklin 1980) and Hong Kong (OngChe 1990). Therefore, growth of new tubules in each reproductive cycle can be expected. Species that have been reported to maintain their after-spawn tubules are *Psolus fabricii* (Hamel et al. 1993) and *Stichopus chloronotus* (Franklin 1980).

The gonadal tubule development observed in *H. leucospilota* did not follow the recruitment model proposed by Smiley (1988) and re-evaluated by Sewell et al. (1997) for other species. Instead of having three groups of tubules that develop subsequently, *H. leucospilota* has only one cohort of tubules that grow concurrently.

The reabsorbtion of gonads in spawned individuals also made this species different to the “tubule recruitment model”. Rather than the gonads being present throughout the year as in the model, there was a period on each reproductive cycle in which they were absent. Furthermore, in the model each group of tubules takes more than one year to reach fecundity, while *H. leucospilota* required less than one year. Instead, the pattern of the studied population showed similarities with three species evaluated by Ramofafia and Byrne (2001).

Reproductive cycle

The population of *H. leucospilota* inhabiting tropical Darwin waters exhibited a seasonally reproductive pattern, with a restricted spawning period of only about two weeks. Developing gonads were estimated to reach maturity in less than one year, inclusive of a 1–2 months resting period between cycles. The reproductive periods of most dendrochirote and aspidochirote species tend to be longer for tropical species. *H. leucospilota* in tropical Darwin harbour did not appear to follow this tropical reproductive pattern. Observations in mid March 2001 at East Point reef revealed three anterior males (A individuals) resulting from fission (see stages in Conand et al. 1997) having fecund testicular tubules. This was an indication of the period of maximum sexual reproductive activity, and spawning was expected to occur shortly after. Spawning was estimated to occur around the last two weeks of April, between new and full moon, when rainfall was at a minimum and the reef was flooded during most of the day. Both the strict period of spawning and the long period of time during which the reef was flooded are factors possibly leading to increased chances of oocytes making contact with sperm. In addition, warmer waters due to the dry season following the period of breeding would facilitate larval development.

In comparison, the same species from tropical southern Taiwan breeds every summer (Chao et al. 1995). In subtropical Heron Island, *H. leucospilota* showed a long spawning season from November to March (Franklin 1980). This period was much longer than the suggested maximum period of two weeks in the investigated population. In the northern hemisphere, in Hong Kong (OngChe 1990) gonads of *H. leucospilota* do not develop synchronously, and the gonad index analysis has been applied to estimate the spawning season to be from August to September. This also occurs in a population from tropical southern Vietnam, which exhibited two peaks, the summer spawning (June–August) being not as intensive and synchronised as the spring spawning (February–March) (Nguyen and Britayev 1992).

Interannual variation in the spawning period probably occurs in the population investigated. This may be caused by variations in environmental conditions. Changes in environmental factors may be responsible for interannual variation in various ways. Stimulation of spawning may involve more than one environmental factor (Conand 1993; Hamel and Mercier 1995). Spawning in *Aslia levefrei* is stimulated by high temperature and light intensity, and in *Holothuria scabra* by changes of salinity (OngChe 1985, Krishnaswamy and Krisnan 1967). In *Holothu-*

ria pulla and *H. coluber*, temperature, monsoon, lunar cycle and chemicals produced by males and females are suggested to be important factors for spawning (Bantula-Batoy et al. 1998).

The importance of sexual reproduction on population

Relative importance of sexual reproduction has been questioned in holothurian populations with intensive fission activity. During the 18-month study, juveniles were hardly found among the fissiparous populations of *H. leucospilota*. This suggests that, unless the larvae were washed away, recruitment from sexual reproduction on the habitat may have been unsuccessful. Considering the lack of juveniles on the reef, well developed gonads in individuals of *H. leucospilota* are probably not a guarantee of recruitment from sexual reproduction.

Maturation of gonads in the *H. leucospilota* of East Point took place during a period of intensive asexual reproduction, which occurred from January to April (Purwati 2001). Furthermore, fission seemed to occur regardless of the maturity stage of the individuals, as anterior individuals having fecund testes were observed dividing. It is possible that fission is more significant in population maintenance of *H. leucospilota* of Darwin waters than sexual reproduction. This raises the question: Does intensive fission prevent recruitment following sexual reproduction, or does failure of sexual recruitment generate intensive fission activity?

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Natural spawning observations of *Pearsonothuria graeffei*

Pradina Purwati[1]

Spawning of *Pearsonothuria graeffei* in its natural habitat was observed during Anambas Expedition 2001. The animals stood up, waving the anterior part of the body slowly, and spilled their gametes into the water column.

Dates and locations: 13 March 2002 on the north-eastern part of Jebung Bay, Jemaja Island (03°15.19'N and 106°13.48'E) and 14 March 2002 on the south western coast of Matak Island (02°52.43'N to 02°54.63'N and 105°50.97'E), Anambas islands, South China Sea.

Depth: down to 32 m

Time of spawning observation: 05:00–06:00 pm

Habitat: reef slope, white sand, a lot of boulders and branching corals (first site); shallow disturbed fringing reef, damage and dead corals (second site).

Other echinoderm on the sites: *Diadema setosum*

***Pearsonothuria graeffei* spawning in the wild**

Natural spawning observation of *Stichopus hermanni*

Aymeric Desurmont[2]

Location: Baie des Citrons, Noumea, New Caledonia (22°15'S and 166°25'E)

Date and time: 12 February 2003, 05:30 pm

Depth: 4 m

Bottom: rocky with small patches of sand and coral

Moon phase: 4 days before full moon

Tide: 1.5 hours after high tide.

Description: One specimen of curryfish (*Stichopus hermanni*), about 50 cm long, was erected on the top of a small rocky pinnacle. It was slowly swaying while releasing dribbles of gametes. No other curryfish was visible in a 15-m radius. Several specimens of other sea cucumber species (*Bohadschia argus, B. vitiensis, Holothuria atra, H. coluber, H. edulis* and *Stichopus chloronotus*) were present in the surroundings, but none was showing signs of reproductive activity. The curryfish kept releasing gametes during the 20 minutes that the observation lasted.

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