

## Fissiparity in *Holothuria leucospilota* from tropical Darwin waters, northern Australia

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### Abstract

Fission was studied in a population of *Holothuria leucospilota* at Darwin harbour. Fission resulted in smaller size anterior portions (A to P ca. 34.81%). A greater number of posterior individuals (P and Pa) may be assumed to mean a greater survival capacity than with anterior individuals (A and Ap). Monthly observations from September 1998 to September 1999 revealed that fission occurred throughout the year at an estimated rate of up to 28% at East Point and 8% at Nightcliff. At East Point, maximum fission occurred in April and coincided with a period of sexual reproduction, when annual precipitation was at a maximum and the reefs were exposed for less than 4 hrs day<sup>-1</sup>. This study suggests that *H. leucospilota* may use fission to maintain population level when sexual recruitment fails.

### Introduction

Townsley and Townsley (1973) reported the occurrence of small individuals of *Holothuria leucospilota* at Fanning Island. This led to the speculation that recruitment of this species is via spontaneous fission. This phenomenon was only confirmed in 1997 when Conand et al. (1997) reported fissiparity in *H. leucospilota* from Reunion Island, Indian Ocean, bringing the total of fissiparous holothurian species to 10 (Purwati 2001).

One common feature among fissiparous holothurians is that the fission potential is not expressed in all habitats. The factors that trigger fission (i.e. those that work locally or specifically) have been debated. When two or more fissiparous species share the same habitat, not all of them undergo fission. At Rongelap Atoll, *H. atra* is triggered to fission, but *H. leucospilota* is not (Bonham and Held 1963). At Heron Island, *S. chloronotus* undergoes fission, but *H. leucospilota* does not (Franklin 1980). This kind of phenomenon also occurs with *H. leucospilota*, *H. atra* and *S. chloronotus*, which share the same habitat at Fanning Island (Townsley and Townsley 1973) and *H. atra*, *S. chloronotus* and *H. edulis*, which were kept under laboratory condition (Uthicke 1997).

This paper aims to determine the seasonal pattern of *H. leucospilota* asexual reproduction at Darwin harbour, and to analyse the results of monthly observations of individuals resulting from fission. Because these *H. leucospilota* populations also undergo sexual reproduction (Purwati and Luongvan 2003), the relative importance of fission will also be evaluated.

### Materials and methods

An investigation of *H. leucospilota* was made at Nightcliff (12°22'45 S and 130°50'45 E) and East Point (12°24'20 S and 130°50'49 E), in Darwin, northern Australia. These areas are dominated by a wet and dry season, and experience semi diurnal tides. Individuals of *H. leucospilota* inhabited intertidal areas of approximately 300 m x 500 m at Nightcliff, and 500 m x 700 m at East Point. Individuals showed patchy distribution over the intertidal reefs, small sandbars (mainly at Nightcliff), and rocky areas (mainly at East Point).

### Population densities

Transect lines were established on Nightcliff's intertidal zone in November 1999. Eight 160-m-long transect lines were placed every 20 m, and each line contained eight circular quadrats (4 m in diameter). Within each circle, the number of individuals was recorded.

East Point reef is a rock formation with a lower area of silt and rubble. Because of this, it was necessary to estimate the population density separately. The lower area experiences two low and high tides a day. Five circular quadrats (4 m in radius) were randomly distributed.

During ebb tide on the rock, individuals were more likely to be trapped in tidepools as only high spring tides cover the entire area. During ebb tides, tidepools of varying size were exposed in the (or intervening with) dry rocky areas. This arrangement of tidepools prevented the use of transect lines. Instead, holothurians from seven standing

tidepools were counted to provide an estimate of the number of individuals per square meter.

### **Individual size and anatomy**

The fresh body weight of 50 individuals was measured using a 500 g Pesola field balance every three months. Only individuals with stiff skin were sampled as they were considered to hold the optimum amount of water in their bodies. Any water expelled from the posterior end was weighed inclusively. As soon as a randomly chosen individual was taken from the water, it was placed in a plastic bag, suspended on the balance. This procedure was used in order to minimise disturbance.

Thirty individuals resulting from fission were dissected to examine the internal organs. Cut was made along the body on the ventral side after being drugged with 10 per cent magnesium sulphate for two to three hours.

### **Fission frequencies**

Fission was monitored monthly from August 1998 to January 2000, during daytime low spring tides. Fifty individuals randomly collected were classified according to Conand and De Ridder (1990) into:

- normal individual (N);
- individual in process of fission (F);
- anterior end (A) or posterior end (P) individual;
- anterior individual with regenerating posterior complex (Ap) or posterior individual with regenerating anterior complex (Pa).

The fission and regenerating rate were then estimated based on formulas by Conand et al. (1997):

- Fission rate (F%): 
$$\frac{(A + P)}{2 \times n} \times 100$$
- Regenerating rate (R%): 
$$\frac{(Ap + Pa)}{n} \times 100$$
- Product of fission (%): 
$$\frac{A + P + Ap + Pa + (2 \times \text{recently fission})}{n} \times 100$$

(n: total number = 50)

## **Results**

### **Population densities and individual size**

The tidepools at Nightcliff were comparatively shallow, with a mean depth at low tide of less than 30 cm. The substratum was silty, and provided numerous shelters due to the presence of dead corals

and rocks. At low tide, the water temperature of the tidepools ranged from 30–36°C in an exposed area, and 28–34°C in shaded crevices. Fewer individuals were found closer to the beach. During low tide on the rock platform at East Point, several pools had deeper (approximately 50–70 cm) and clearer water with relatively coarser sand grains and less silt on the bottom than those at Nightcliff. The water temperature at spring low tide ranged from 30–38°C. Otherwise, the water temperature varied between 30 and 36°C.

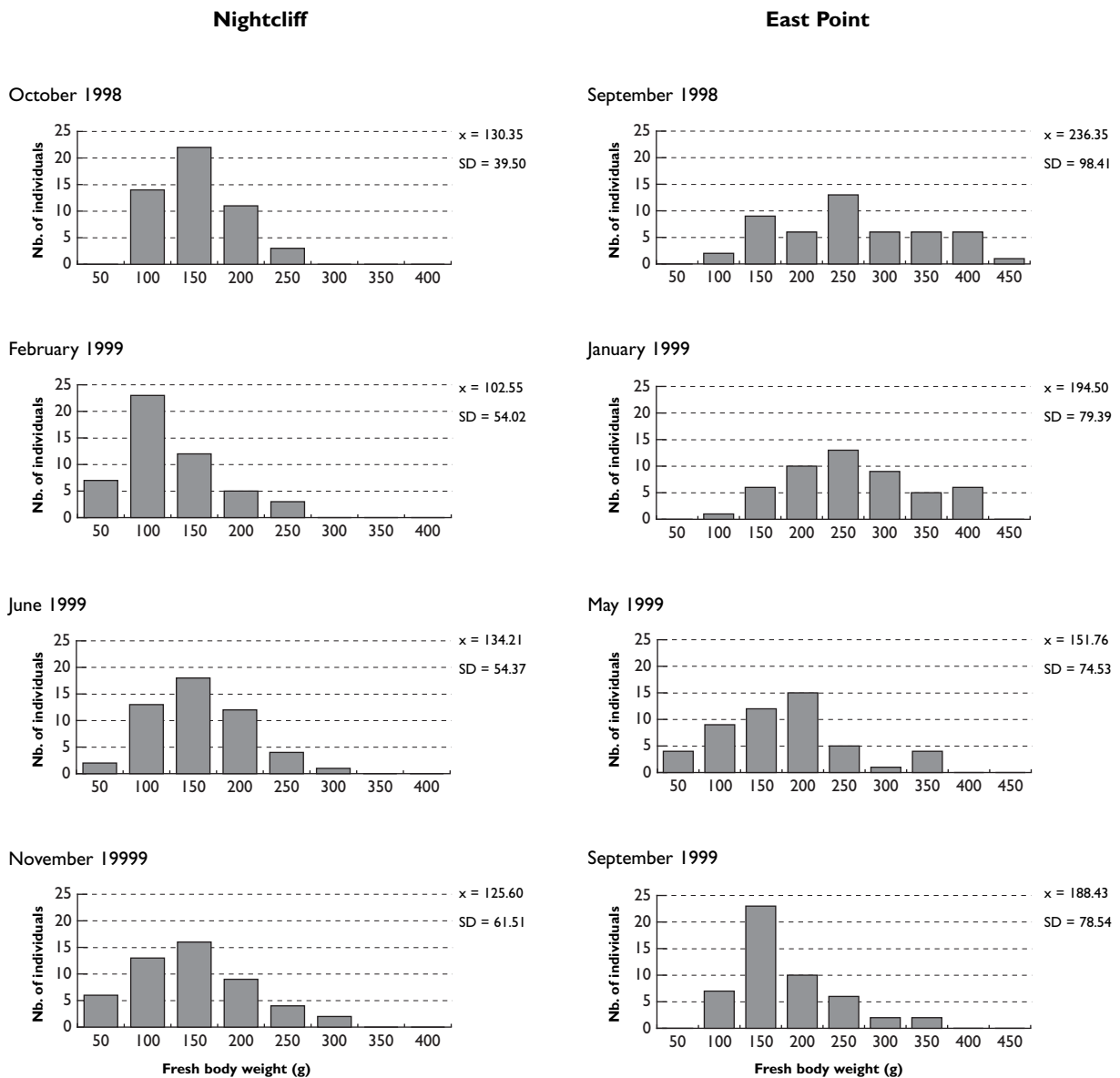


**Figure 1. Individual undergoing fission at Nightcliff**

The density of holothurians at Nightcliff was estimated to be 0.054 individual m<sup>-2</sup>, while random sampling over the lower area of East Point gave an estimate of 0.077 individual m<sup>-2</sup>, and within tidepools on rocky platforms, density was 0.290 individual m<sup>-2</sup>.

At Nightcliff, the largest individuals were between 200 and 225 g in drained body weight, only rarely reaching 275 g. More than 50% of collected individuals were 50–125 g, and individuals less than 50 g were more common there than at East Point. The size range at East Point was greater than that at Nightcliff, with the maximum size of individuals exceeding more than 450 g. Small, up to 50 g, individuals appeared in May 1999, which coincided with a period following intensive fission (Fig. 2). A “T test” of mean individual sizes (measured in successive sampling sessions in the two areas) showed that individuals at Nightcliff were significantly smaller (test,  $P < 0.05$ ). Exceptions occurred between June (at Nightcliff) and May (at East Point) ( $P = 0.1514$ ). This coincided with a high fission rate at East Point.

Most successive sampling sessions at Nightcliff showed significant difference in mean individual size. This may be a consequence of a relatively stable fission rate. At East Point, there was no significant difference, except between January and



**Figure 2.** *H. leucospilota* size composition in Nightcliff and East Point

September 1999, which coincided with a pre- and post-intensive fission period (Fig. 2).

Ten out of 29 individuals kept in aquaria underwent fission from August–September 1998, March–April 1999, and January 2000. In order to estimate the position where the body split prior to division, the fresh body weight of these fission animals was recorded (Table 1).

**Fission frequencies**

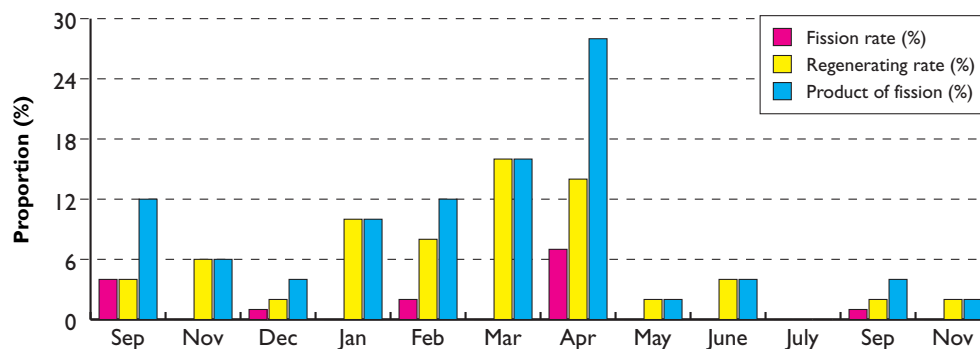
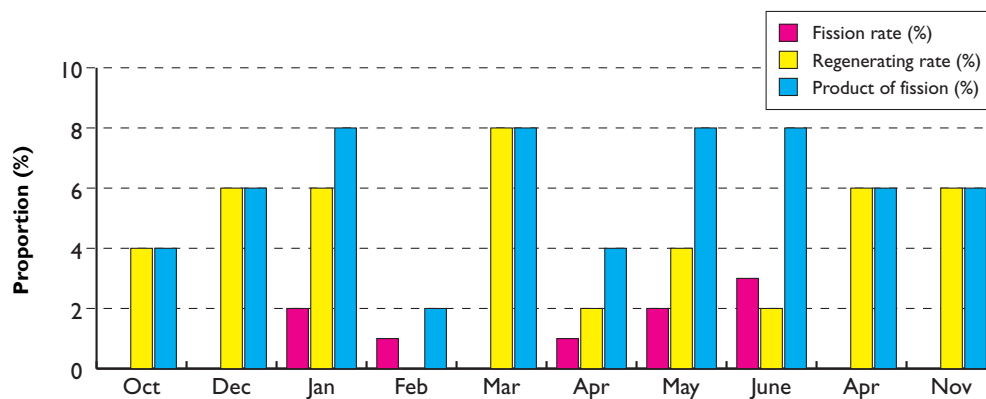
All fission categories were present every month except July at East Point (Fig. 3). The average fission product was 8.33% of the sampled population, with

a maximum value of 28% at East Point, and 6% with maximum of 8% at Nightcliff. The frequency of the regenerating rate increased in January and reached a maximum in March and April at East Point. At Nightcliff on the other hand, the regenerating rate went up and down slightly, between 2 and 6%, with a maximum of 8% in March.

Recently fissioned individuals were always found to be less numerous than regenerating individuals. Between regenerating categories, the number of Pa was higher (2.0% at East Point and 1.6 at Nightcliff) than Ap (0.9% at East Point and 0.6% at Nightcliff), indicating a possible difference in survival rates between the two ends.

**Table 1.** Proportion, in fresh weight (g), of A and P individuals that divided transversally in the aquarium (n = 10).

Date	Mouth end A (g)	Anal end P (g)	A to P (%)	A/A+P (%)
Aug. 98	17.40	143.65	12.11	10.80
Sep. 98	61.14	101.05	60.50	37.70
	24.54	63.07	38.91	28.01
Mar. 99	30.39	87.04	34.91	25.88
	32.07	102.93	31.16	23.76
	29.74	98.72	30.13	23.15
	22.57	89.09	25.33	20.21
Apr. 99	29.43	73.49	40.05	28.60
	43.49	101.17	42.99	
	30.06			
Jan. 00	50.03	156.28	32.01	24.25
Average:	34.08	101.65	34.81	25.24
SD:	13.48	28.70	12.56	6.98

**a) East Point**

**b) Nightcliff**

**Figure 3.** Proportion of fission products

### Anatomical variations

Specimens that recently underwent fission showed no open wound on either body end. Mouth-part individuals (A and Ap) were always with an intact buccal complex, and anal-part individuals (P and Pa) with an intact anal complex. The intestines of most specimens were empty, indicating they had stopped feeding. Muscle bands and intestines appeared to grow earlier than other organs, both in anterior and posterior end individuals (Table 2).

### Discussion

#### The phenomenon of fission

Multiple fission was not observed in the *H. leucospilota* populations studied. Likewise, it has not been found among other fissiparous species of Aspidochirota or Dendrochirota, even though six individuals of *H. parvula* resulting from fission have been reported without both anal and anal parts (Emson and Mladenov 1987). Fission in *H. leucospilota* did not seem to create an open-ended body, or spoil any internal organs. The mechanism of twisting and constricting occurring prior to fission was

suggested to promote tight closure of the fission area immediately after the body separated. In the laboratory, fission was not observed, although it was possible it occurred during the night, as has been suggested for *S. chloronotus* (Uthicke 1997).

Regeneration of internal organs began not long after the external wound healed. Muscle bands regrew in individuals of A and P, and together with the intestine reached a single point at the healed wound of either anterior or posterior ends. The empty intestines indicated no feeding activities. In Ap individuals of the same species inhabiting Reunion Island, the anus has been suggested to develop prior to the lengthening of the intestine, forming an “s” shape (Conand et al. 1997). This may take considerable time.

Anal end portions seem to have a better survival rate than mouth end individuals. The same was observed for *H. leucospilota* on Reunion Island (Conand et al. 1997), and other species including *H. atra* in Nanwan, Taiwan (Chao et al. 1993) and *S. chloronotus* on the Great Barrier Reef (Conand et al. 2002). However, in *H. parvula* from Fort St. Catherine, Bermuda, both portions of the body

**Table 2.** Anatomical condition of dissected individuals

Fission categories (morph)	Anatomical conditions
A 10 specimens in total	<ul style="list-style-type: none"> <li>• Anterior complex intact.</li> <li>• Intestines empty with yellow or greenish colour; short with rupture at posterior end or running straight to posterior-blind-end, attached to inner body wall joining tips of five pairs of muscle bands while cloaca and anal aperture absent. One specimen was observed to have small amounts of fine-grained sand at distal portion of intestine, which were not attached to the inner side of posterior end of integument. Only on occasion did the muscle bands not reach the posterior blind end.</li> <li>• Respiratory trees: only distal portion of right side.</li> <li>• Haemal network: present in several individuals.</li> <li>• Gonad: proximal parts of tubules hanging on gonad basis observed in one individual</li> </ul>
P 9 specimens in total	<ul style="list-style-type: none"> <li>• Anal/posterior complex intact.</li> <li>• Intestines thin, yellow or greenish; long enough to create “s” shape with the end free and ruptured; or straight with posterior end in association with inner wall of integument at anterior-blind-end, joining muscle band ends; mouth and other buccal compartment not developed.</li> <li>• Gonad: not found with gonad basis. A single individual maintained distal parts of gonad tubules free in body cavity</li> </ul>
Ap 5 specimens in total	<ul style="list-style-type: none"> <li>• Calcareous ring and buccal area intact.</li> <li>• Intestine: always found reaching inner side of posterior-blind-end. Most with empty intestines.</li> <li>• Cloaca: membrane-like structure found in two specimens; absent in others</li> <li>• Gonad: not found. (One specimen found with complete visceral organs, except gonad.)</li> </ul>
Pa 6 specimens in total	<ul style="list-style-type: none"> <li>• Calcareous ring: might be absent or present, tentacles may not be obvious.</li> <li>• Intestines: distal portion always reached inner integument surface; relatively long, might be in “s” shape.</li> <li>• Haemal network: always present</li> <li>• Gonad: only one female and one male with fully-grown gonad (orange in female and creamy in male, but tubules relatively small in number and dimension). One specimen found with complete viscerae, except gonad (integument at anterior end was pale and print of tube feet arrangement remained).</li> </ul>

have similar rates of survival (Emson and Mladenov 1987). Behaviours such as *H. leucospilota* anchoring its body using the posterior end (Bonham and Held 1963) may reduce the probability of anal end individuals (P and Pa) being washed away, while mouth end individuals may be more easily swept away and accessible to predators.

This study confirms that the body area where fission occurs is specific to each fissiparous species. Fission produced smaller mouth end individuals, similar to that found at Reunion (Conand et al. 1997) and Fanning islands (Townesley and Townesley 1973). In *H. atra*, the mouth end part is smaller (44%) than the anal end portion (Chao et al. 1993; Conand 1996), whereas in *S. chloronotus* the mouth end part is slightly larger than the anal end portion (Conand et al. 1998). In *H. parvula* (Kille 1942; Emson and Mladenov 1987), and *H. surinamensis* populations (Crozier 1917), the area of constriction is in the middle of the body.

In the present study, the gonadal base belonged to the mouth end individual (A), which differs from Conand et al. observations (1997). The anal end individual (P) held the distal parts of gonad tubules, which were located freely within the body cavity. Interestingly, the gonadal tubule at both ends was blind and retained full-grown oocytes. It seemed that gonadal tubules had been somehow prepared to break down before the body divided morphologically into two different portions. Whether these tubules were absorbed later remains unknown.

### **Fission frequency**

Fission occurred throughout the year in populations of *H. leucospilota* at both sites. Compared with the continuous fission of the *H. parvula* population in Bermuda (Emson and Mladenov 1987) or to the seasonal fission of *S. chloronotus* at Reunion Island (Conand et al. 1998), the fission period for *H. leucospilota* was shorter. Higher frequencies of fission in East Point coincided with high rainfall, from December to March. During this period, low spring tides totally exposed the reef between 1100 and 1600 hr (the same was true for Nightcliff), which confirmed Ferns' (1995) observations. During these months, desiccation is more intense, direct solar radiation and water temperature are greater, while salinity is lower. One or a combination of these conditions may trigger fission.

Spontaneous fission of *H. leucospilota* also occurred at the laboratory where the above environmental conditions were absent. Two other fissiparous species, *S. chloronotus* and *H. edulis* have been reported to divide under laboratory conditions (Uthicke 1997). Unless unknown stimulating fac-

tors were present at the laboratory, it is possible that the individuals have been naturally stimulated before being transferred to the laboratory. In this case, triggers would require an "incubation time" before individuals act on the stimuli. However, it is unlikely, as fission still occurred two to three weeks after the individuals had been kept in the laboratory. Under-nourishment may also be considered as one of the possible fission stimulators, as food was not given during rearing. Again, it was unlikely to be the fission triggering factor for *H. atra*, a species usually living in eutric areas (Conand 1996), during experiments where more food was made available for sediment feeders (Uthicke 1997). The empty intestines and blind ended fractions of gonadal tubules in recently divided individuals may offer evidence that internal organs prepared for division before the body splits.

### **Role of fission in the population**

Current investigation conforms to general characteristics of fissioning populations: high-density populations of mostly small individuals (Chao et al. 1993; Conand et al. 1998; Uthicke 1997). When fission produces small individuals, the habitat is able to accommodate larger numbers, because biomass is related to habitat capacity (Uthicke 2001). In comparison, the population of *H. leucospilota* at Heron Island, in which fission is absent, has been reported to have wet body weight between 10 and 1200 g, with a density of 0.031–0.245 ind. m<sup>-2</sup> (Franklin 1980).

Several researchers agree that a high rate of fission, and a low recruitment of juveniles produced through gamete fertilisation, may indicate that fission is important in sustaining local populations. Fission may play a role in either maintaining population density (Emson and Mladenov 1987; Conand et al. 1998) or increasing it (Chao et al. 1993; Chao et al. 1994). This seems to be the case with the Nightcliff and East Point populations, where small, normal individuals less than 10 cm long or weighing less than 10 g of fresh body weight were hardly found.

Fissiparous species use their asexual reproduction ability only in certain habitats. But, there is no report of a fissiparous population that does not develop gonads (that are not able to reproduce sexually). This may demonstrate that fission is a secondary strategy, operating when sexual reproduction fails.

Naturally, every population attempts to reach optimum density in its habitat (Uthicke 1997). In fissiparous holothurians, both asexual and sexual reproductions may occur simultaneously in order to



maintain population. When sexual reproduction is incapable of attaining the optimum population density (because of insufficient number of breeders or failure during embryonic and larval stage), then perhaps, fission potential would be generated to function. In this case, fission would act to compensate unsuccessful sexual reproduction. Indeed, this hypothesis requires further investigation. However, if the dual role of fission in population maintenance is acceptable (as a replacement for and as supplemental to sexual reproduction), then it is unlikely that fission reduces sexual reproductive activity as suggested by Emson and Mladenov (1987), Chao et al. (1994) and Conand (1996).

Sexual reproductive efficiency may decrease when individuals become smaller in size due to self-division. And, development of gonads in the population may not guarantee success of sexual recruitment, as failure may occur after the gametes are released. When the environment becomes risky for sexual recruitment, fission seems to have the potential to compensate the failure, as individuals produced by fission have already adapted to the parent habitats, as well as to predatory avoidance (Emlet et al. 1987). In such cases, fission becomes more effective in population upholding.

Unlike *S. chloronotus* populations on the Great Barrier Reef and Reunion Island, for which fission and spawning occur at different periods (Conand et al. 2002), *H. leucospilota* populations showed a limited spawning activity (Purwati and Luong-van 2003) and an important fission activity taking place during the same month. Does it indicate that fission is the main strategy of population upholding in the local habitat of Darwin harbour? Whatever the importance of asexual reproduction in this *H. leucospilota* population, it maintains a population with a sex ratio of 1:1 (Purwati and Luong-van 2003). This means that fertilisation can still be accommodated, or, in other words, that the ability for this *H. leucospilota* population to reproduce sexually is not affected by fission. In *S. chloronotus*, a high fission rate produces a population with more males than females (Conand et al. 2002) — which may imply that males have a higher potential to undergo fission. As a consequence, during spawning events, there is a limited number of eggs in the water column and chances of a successful sexual reproduction are limited. Therefore, it is true that fission reduces the sexual reproductive ability in this population, as is the case with *H. atra* (Conand 1996).

### **The possible usage of fission potential**

The beche-de-mer trade involves 15–20 holothurian species, including at least four fissiparous species. Fission potential of these species could be

used to accelerate restocking and population enhancement. Reichenbach and Holloway (1995) have conducted an interesting fission experiment on several commercial holothurian species. Rubber bands were put around specimens to induce fission. The technique successfully stimulated binary division, but only *S. chloronotus* and *Thelenota ananas* had a high survival rate for both ends. *A. miliaris* and *S. variegatus* were able to develop the anterior part of anal end individuals, despite the fact that the last three-mentioned species are not known to divide naturally in the wild. Since each fissiparous species has a particular fission point, rubber bands placed correctly may increase the survival rate of both portions. This, in turn, may increase the chances of success of a sea cucumber stock enhancement programme based on reproduction by induced fission.

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