Asexual reproduction by fission of a population of *Holothuria hilla* (Lesson 1830) at One Tree Island, Great Barrier Reef, Australia

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Introduction

Asexual reproduction through fission is a feature of many of the most abundant aspidochirote holothuroids in the Indo-Pacific region (Uthicke 1997, 2001). Fissiparious species split in half and the two halves regenerate to make complete individuals. Thus far, six *Holothuria* and two *Stichopus* species are known to be fissiparious and asexual reproduction appears to be a major form of reproduction used in maintaining populations (Ebert 1978; Harriott 1982; Conand and de Ridder 1990; Chao et al. 1994; Conand et al. 1997; Uthicke 2001). All aspidochirotids for which asexual reproduction has been described also reproduce sexually and have dispersive larvae. Spawning of aspidochirotids occurs at variable times of the year for different species, with spawning usually in summer (e.g. Conand 1993; Guzman et al. 2003; Ramofafia et al. 2000) however exceptions such as the winter spawning of *H. whitmaei* have been observed (Conand 1981; Shiell and Uthicke 2006). Asexual and sexual reproduction are both important in the maintenance and genetic structure of populations of tropical aspidochirotids (Conand et al. 2002).

In this study we investigated the asexual and sexual reproduction in a population of *Holothuria hilla*. *H. hilla* is a soft-bodied holothuroid that is widespread in the Indo-Pacific region (Kerr 1994; Rowe and Gates 1995). It has a distinctive light-brown body wall with light-yellow to white papillae. *H. hilla* tends to be nocturnal and is found under coral rubble or on reef flats region emerging at dusk to feed (Lawrence 1979; Kerr 1994). On midshelf reefs of the Great Barrier Reef, densities of 82–220 ind ha^{-1} have been observed, with lower numbers on outer shelf reefs (Hammond et al. 1985).

Materials and methods

One Tree Island (OTI; located at 23°30′S, 152°05′E) is in the Capricorn Bunker Group of the Great Barrier Reef. Asexual reproduction in *Holothuria hilla* was monitored in samples collected at 6 to 7 week intervals from March 2005 to November 2006. No samples were collected from December to February. *H. hilla* were collected at low tide from under coral rubble from the inlet on the east side of OTI. The length of each *H. hilla* was measured with a ruler and weighed using a 1-kg Super Samson spring balance to the nearest 5 g. Each specimen was examined externally for signs of fission. They were categorised into five stages of fission and regeneration according to Conand (1996): (1) W = whole or undivided specimen, (2) A = an anterior specimen with an existing mouth at one end and a closed wound at the other, and (3) P = a posterior specimen with an anus at one end and a closed wound at the other. All specimens were returned to the collection site. The proportion of anterior and posterior fission products were compared by a paired t-test. The location of *H. hilla* at night was investigated in night snorkels in July 2004 and November 2005 from approximately three hours after sunset.

In November 2006 we examined five individuals for the presence of gonads. Histology was used to document gamete development and to define the stage of gonad maturation. The gonads were fixed in formalin and stored in 70% ethanol. For histology, gonad sections (7 µm) were stained with Mayer's haemotoxylin and eosin (H/E), and the slides were examined by light microscopy. Stages of gametogenesis were classified into one of the following five categories: (1) Indeterminate — unable to define whether the specimens are male or female as no gametes is evident; (2) Growing — developing eggs and sperm in the gonad; (3) Mature — fully developed eggs or sperm were present in the tubules. This was seen by a great volume of eggs or sperm packed tightly inside the tubules; (4) Partly spawned — eggs or sperm seen in the tubules and maybe loosely arrayed due to recent release of its gametes. Some gonad gametes were still growing to maturity; and (5) Spent — tubules were found to be empty. Residual oocytes or sperm may be present. Brown bodies usually seen in association with spent tubules.

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Results

H. hilla was locally abundant in the inlet adjacent to One Tree Reef. During the day it was found under coral rubble, emerging at night to feed. The maximum length of whole individuals was 15–20 cm and maximum weight was 75–80 g. For fission products, the maximum length and weight was 10–15 cm and 45–50 g, respectively.

Incidence of asexual reproduction and growth

At the beginning of the study, in March 2005, fission products of *H. hilla* were evident in the population. The body of *H. hilla* is light brown distinct with white or yellow papillae. *H. hilla* that have undergone fission are easily distinguished because the regenerating region of the body is lighter in colour and smaller in diameter than the original body (Fig. 1). The various stages of fission and regeneration were found within all sampling times. However, we did not encounter individuals in the process of fission. Although fission occurs year round, it appears to be more prevalent in cooler months (May–August). Over the 20-month study (Fig. 2a), fission was most prevalent in June 2005 and July 2006 when fission products were 59% of the specimens collected. The incidence of fission was lowest in November 2005 (0%) and 2006 (14%). The presence of anterior and posterior fission products in the population varied over time (Fig. 2b), but the proportion of these individuals did not differ (df = 9, t = 0.246, p >0.05).

Figure 3 shows the frequency distribution of weight for *H. hilla*. The weight range of whole *H. hilla* was 10–210 g while fission products were 10–110 g. Large non-regenerating *H. hilla* were 5–35 cm in length and fission products were 5–25 cm in length (Fig. 4). The minimum mean weight was found in March 2005 at 42.3 g (SE = 8.38 g, n = 24). The heaviest specimens were found in November 2006 (mean = 113.5 g, SE = 12.2, n = 26). This also corresponds with the highest proportion of whole non-regenerating individuals. In 2005, the mean weight and length of *H. hilla* in the populations increased from a low of 42.2 g and 14 cm respectively in March, to 80 g and 22 cm respectively in November. Similarly, in 2006 mean weight and length was 49.8 g and 14 cm in April and 113.5 g and 20 cm in November. This represents a gain in weight of ca. 56% in 2005 and 89% in 2006 over 6–7 months, equivalent to a weight gain of 3.14 and 5.3 g per month respectively. If individuals only split once during this period, the *H. hilla* approximately doubled in weight post fission.

Sexual reproduction

The gonad of *H. hilla* is a single tuft with numerous branching tubules attached to the gonad basis on the anterior body wall. The sex of the gonads for the majority of the specimens was difficult to determine macroscopically but histological examination showed all five specimens had gonads: one was female, one was male and the remaining were of spent (with no remaining gametes) or indeter-

Figure 1. Fission products of *Holothuria hilla*.
Posterior fission product (top) and anterior fission product (bottom).
Arrows show the regenerating regions. Scale = 1:2

minate stages (Figs 5A–F). The sex of the specimen could not be determined in the indeterminate specimens. Females were distinguishable as spent ovary tubules contained a few remaining oocytes (Figs 5A and B). The oocytes were on average 88.19 µm in diameter (n = 30, SD = 11.52). Indeterminate and spent specimens had an empty lumen and highly reduced tubules with a wrinkled appearance (Figs 5C–F). No gametes were present in the indeterminate and spent specimens.

Discussion

This is the first documented study of asexual reproduction in *Holothuria hilla*. As for other fissiparious holothuroids, *H. atra* and *Stichopus chloronotus* on the Great Barrier Reef and elsewhere (Conand 1996; Conand et al. 1997; Uthicke 1997; Conand et al. 2002), asexual reproduction in *H. hilla* predominates in the cooler months. Populations of *H. hilla* in Hawaii also undergo fission (Discipline of Anatomy and Histology, Bosch Insitute, University of Sydney, pers. comm.). This is a popular species in hobby aquaria and is also known to undergo fission in aquaria (Robert Toonen, Hawaii Institute of Marine Biology, University of Hawaii Toonen, University of Hawaii, pers. comm.).

Fission in *H. hilla* was prevalent for eight months of the year over the cooler months sampled (March–October) from autumn to spring. The highest incidence of fission in *H. hilla* in June and July coincided with mid-winter, similar to that documented for populations of *H. atra* and *S. chloronotus* on OTI and elsewhere (Harriott 1982; Conand 1996; Uthicke 1997, 2001; Lee 2005). Fission is considered to play

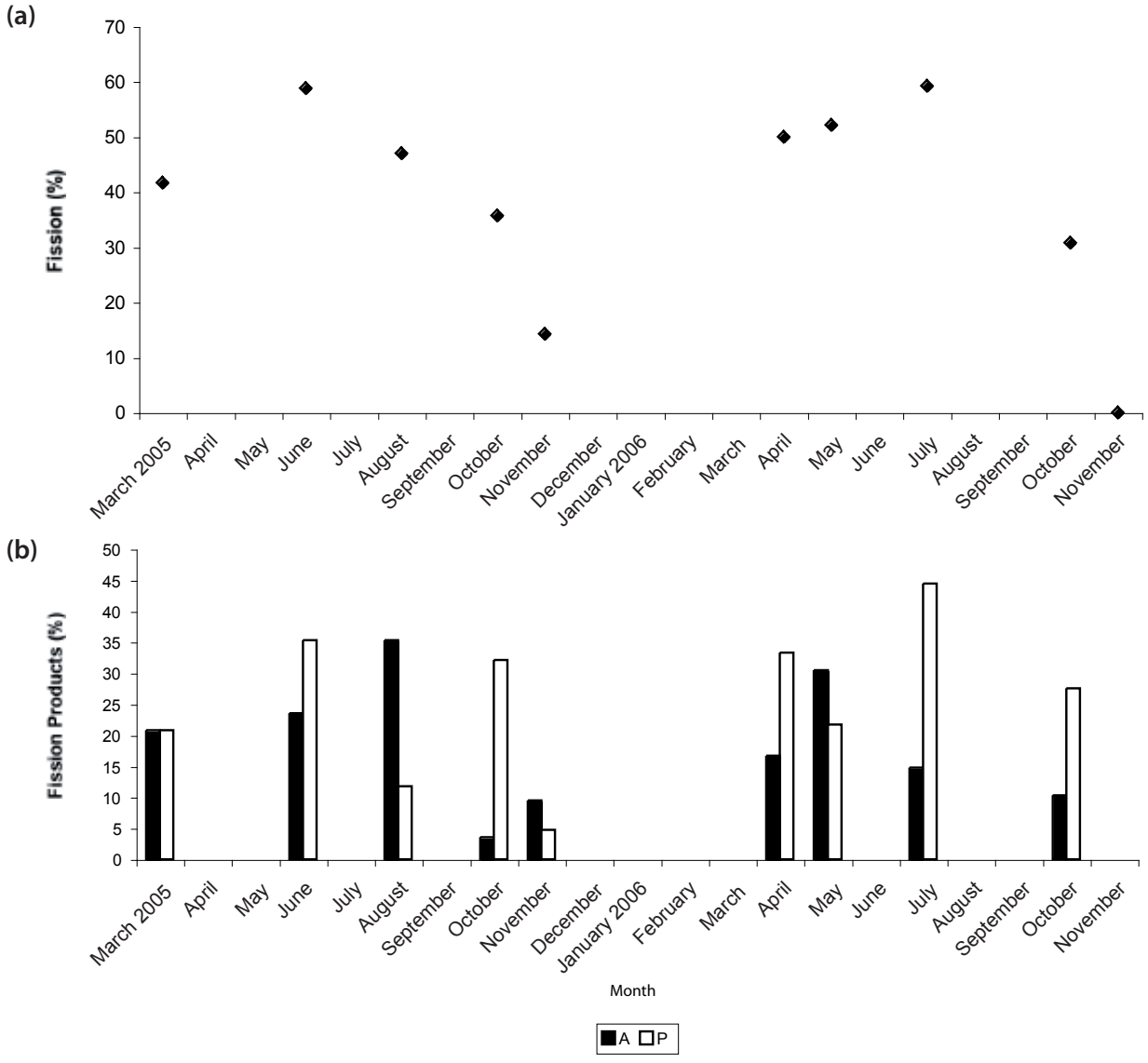

Figure 2. The incidence of asexual reproduction of *Holothuria hilla*:
(a) Percentage of fission in each sample.
(b) Percentage of anterior (black) and posterior (white) fission products in each sample.

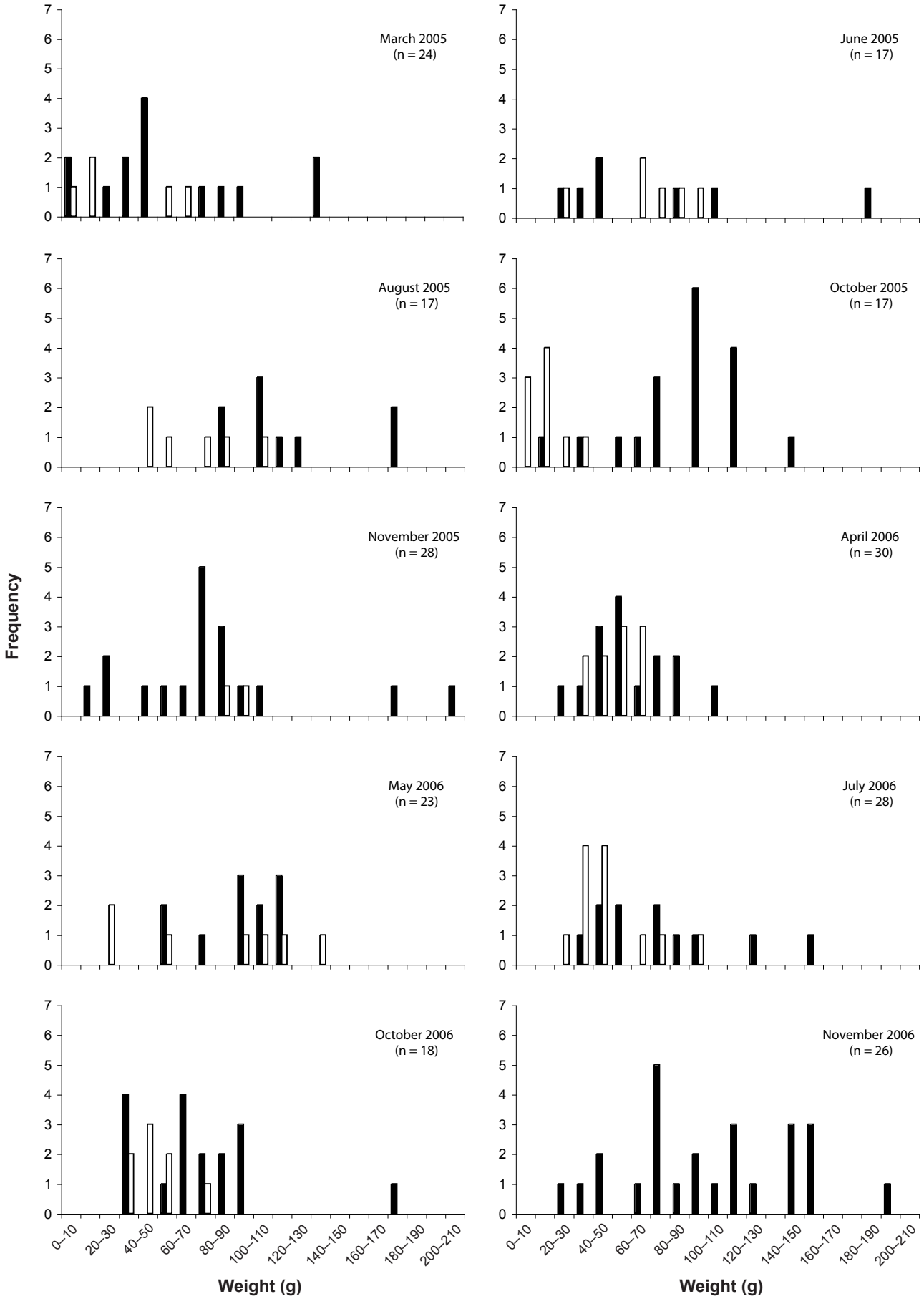

Figure 3. Weight frequency distribution of *Holothuria hilla* over 20 months. Whole individuals (black) and fission products (white). Upper size limit for each category given.

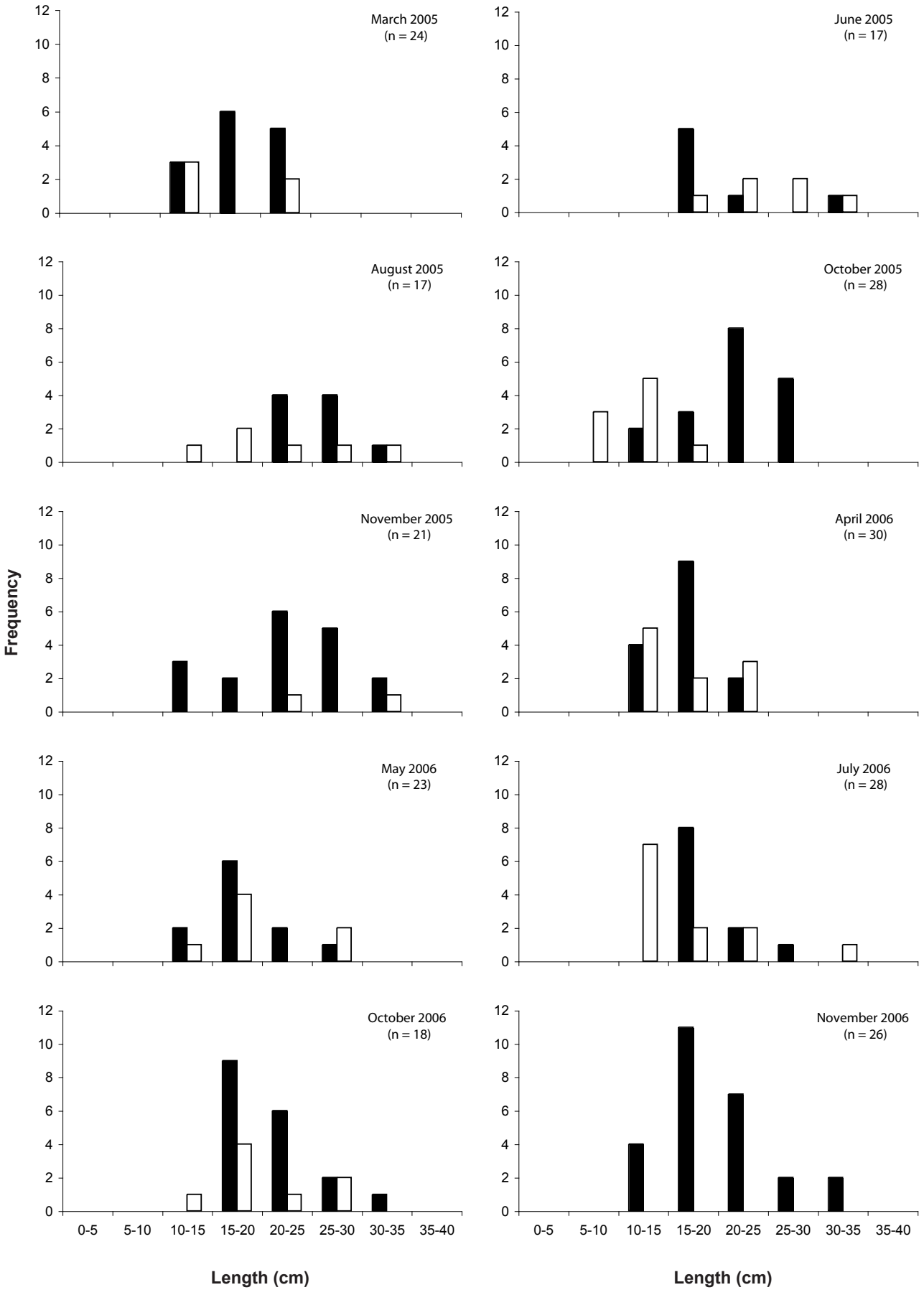

Figure 4. Length frequency distribution of *H. hilla* over 20 months. Whole individuals (black) and fission products (white). Upper size limit for each category given.

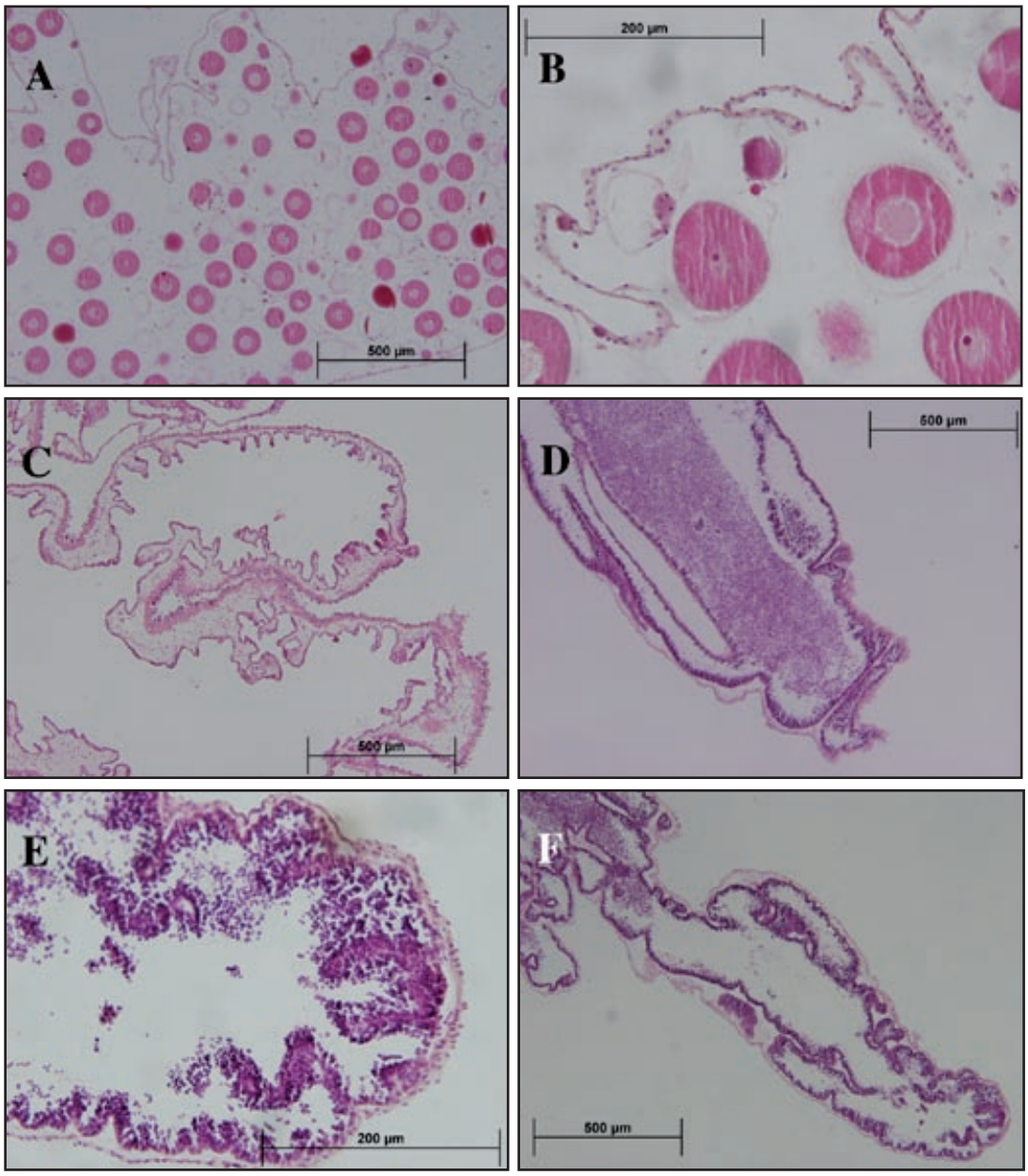

Figure 5. *Holothuria hilla*.
A, B: Spent ovaries; shrinkage of tubules occurring and few oocytes (O) present.
C–F: Spent and indeterminate specimens.

a role in maintaining populations of several Indo-Pacific holothuroids by compensating for mortality and migration (Ebert 1978; Harriott 1982; Chao et al. 1994; Uthicke 2001). This may also be the case for the *H. hilla* population at OTI, but data on population density over time are required to address this.

The mean weight of the population of *H. hilla* increased from March to November in both years. Because fission in *H. hilla* occurs over a long period of time (up to eight months) it is not clear if this weight increase was due to regeneration post one fission event in individuals. As suggested for other fissiparous holothuroids (Uthicke 2001), *H. hilla* may grow to a minimum length and weight size before fission occurs. The approximate doubling in size as the *H. hilla* regenerate and grow post fission is similar to that seen for *H. difficilis* and *H. atra* at OTI and elsewhere (Lee et al. in review).

Fission appears to be suppressed in *H. atra* and *S. chloronotus* in the warmer months, perhaps in association with gonad development and a shift towards sexual reproduction. This may also be the case for *H. hilla*. However, in this study, the stage of maturation of the gametes of *H. hilla* could not be determined as one sample contained gametes remaining from spawning, or gametes on their way to becoming mature, and remaining samples were indeterminate or spent. A cursory look for gonads of *H. hilla* dur-

ing the fission season indicated that they were not present (Maria Byrne, Discipline of Anatomy and Histology, Bosch Insitute, University of Sydney, pers. obs.). Fission products of *H. atra* have been known to contain gonads of all stages of development and have been proposed to regenerate two months after fission (Doty 1977; Uthicke 1997, 1998). Further studies would be required in order to understand the complete reproductive cycle of *H. hilla*.

References

Chao S.M. Chen C.P. and Alexander P.S. 1994. Reproduction and growth of *Holothuria atra* (Echinodermata: Holothuroidea) at two contrasting sites in southern Taiwan. Marine Biology 119:565–570.

Conand C. 1981. Sexual cycle of three commercially important holothurian species (Echinodermata) from the lagoon of New Caledonia. Bulletin of Marine Sciences 31(3):523–544.

Conand C. 1993. Reproductive biology of the characteristic holothurians from the major communities of the New Caledonia lagoon. Marine Biology. 116:439–450.

Conand C. 1996. Asexual reproduction by fission in *Holothuria atra*: variability of some parameters in populations from the tropical Indo-Pacific. Oceanologica Acta 19 (3–4):209–216.

Conand C. and De Ridder C. 1990. Reproduction asexuée par scission chez *Holothuria atra* (Holothuroidea) dans des populations de platiers récifaux. p. 71-76. In: De Ridder, Dubois, Lahaye et Jangoux (eds). Echinoderm Research. Balkema, Rotterdam.

Conand C., Morel C. and Mussard R. 1997. A new study of asexual reproduction in holothurians: Fission in Holothuria leucospilota populations on Reunion Island in the Indian Ocean. SPC Beche-de-Mer Information Bulletin 9:5–11.

Conand C., Uthicke S. and Hoareau T. 2002. Sexual and asexual reproduction of the holothurian *Stichopus chloronotus* (Echinodermata): a comparison between La Reunion (Indian Ocean) and east Australia (Pacific Ocean). Invertebrate Reproduction and Development 41(1–3):235–242.

Doty J.E. 1977. Fission in *Holothuria atra* and holothurian population growth. MSc thesis. University of Guam.

Ebert T.A. 1978. Growth and size of the tropical sea cucumber *Holothuria* (*Halodeima*) *atra* Jager at Enewetak Atoll, Marshall Islands. Pacific Science 32:183–191.

Guzman H.M., Guevara C.A. and Hernandez I.C. 2003. Reproductive cycle of two commercial species of sea cucumbers (Echinodermata: Holothuroidea) from Caribbean Panama. Marine Biology 142:271–279.

Hammond L.S., Birtles R.A., Reichelt R.E. 1985. Holothuroid assemblages on coral reefs across the central section of the Great Barrier Reef. Proceedings of the Fifth International Coral Reef Congress, Tahiti 5:285–290.

Harriott V. 1982. Sexual and asexual reproduction of *Holothuria atra* Jaeger at Heron Island Reef, Great Barrier Reef. Australian Museum Memoir. No. 16:53–66.

Kerr A.M. 1994. Shallow water holothuroids (Echinodermata) of Kosrae, Eastern Caroline Islands. Pacific Science 48(2):161–174.

Lawrence J. 1979. Numbers and biomass of the common Holothuroids on the Windward reef flat at Enewetak atoll, Marshall Island. Proceedings of the European Colloquium on Echinoderms, Brussels.

Lee J. 2005. Asexual reproduction in four species of tropical aspidochirotid holothuroids at One Tree Island, Great Barrier Reef. Sydney, University of Sydney.

Lee J., Byrne M. and Uthicke S. (in review). Asexual and sexual reproduction in the aspidochirotid sea cucumber *Holothuria difficilis* at One Tree Island, Great Barrier Reef. In: Echinoderms, New Hampshire. L. Harris (ed). Taylor and Francis Group, London.

Ramofafia C., Battaglene S.C., Bell J.D. and Byrne M. 2000. Reproductive biology of the commercial sea cucumber *Holothuria fuscogilva* in the Solomon Islands. Marine Biology 136:1045–1056.

Rowe F.W.E. and Gates J. 1995. Echinodermata. In: Wells. A. (ed). Zoological catalogue of Australia. Vol. 33. Melbourne: CSIRO, Australia. Xiii. 510 p.

Shiell G.R. and Uthicke S. 2006. Reproduction of the commercial sea cucumber *Holothuria whitmaei* (Holothuroidea: Aspidochirotida) in the Indian and Pacific Ocean regions of Australia. Marine Biology 148(5):973–986.

Uthicke S. 1997. Seasonality of asexual reproduction in *Holothuria (Halodeima) atra*, *H. (H.) edulis* and *Stichopus chloronotus* (Holothuroidea: Aspidochirotida) on the Great Barrier Reef. Marine Biology 129:435–441.

Uthicke S. 1998. Respiration of *Holothuria (Halodeima) atra*, *Holothuria (Halodeima) edulis* and *Stichopus chloronotus*: Intact individuals and products of asexual reproduction. p. 531 – 536. In: Mooi R. and Telford M. (eds). Echinoderms. Rotterdam: Balkema.

Uthicke S. 2001. Influence of asexual reproduction on the structure and the dynamics of *Holothuria (Halodeima) atra* and *Stichopus chloronotus* populations of the Great Barrier Reef. Marine Freshwater Research 52:205–215.