References

CONAND, C. (1993). Reproductive biology of the holothurians from the major communities of the New Caledonian Lagoon. Mar. Biol. 116: 439–450.

KRISHNASWAMY, S. & S. KRISHNAN (1967). A report on the reproductive cycle of the holothurian *Holothuria scabra* Jaeger. Curr. Sci. 36: 155–156.

ONG CHE, R. G. & E. D. GOMEZ (1985). Reproductive periodicity of *Holothuria scabra* Jaeger at Calatangan, Batangas, Philippines. Asian Mar. Biol. 2: 21–29.

TANAKA, Y. (1958). Seasonal changes occurring in the gonad of *Stichopus japonicus*. Bull. Fac. Fish. Hokkaido University, 9: 29–36.

TUWO, A. & C. CONAND (1992). Reproductive biology of the holothurian *Holothuria forskali* (Echinodermata). J. Mar. Biol. Ass. U.K. 72: 745–758.

TUWO, A & C. CONAND (1996). Commercial holothurians in Southwest Sulawesi (preliminary observations). Torani 6 (2): 129–134.

Asexual reproduction parameters and the influence of fission on a *Holothuria atra* sea cucumber population from a fringing reef on Reunion Island (Indian Ocean)

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Introduction

Holothuria atra is one of the most common sea cucumbers of intertidal zones in the tropical Indian-Pacific region. Asexual reproduction through transverse fission is a biological phenomenon which has already been studied, most notably in the south of the island of Taiwan (Chao & Chang, 1989; Chao et al., 1994), on the reefs of New Caledonia (Conand, 1989) and on the Great Barrier Reef in Australia (Harriot, 1982; Uthicke, 1994; Uthicke, 1997).

On Reunion Island, *H. atra* can be found throughout the fringing reef of the St Gilles/La Saline reef system, where it varies in size from 10 to 30 cm and in weight from 10 to 220 g. It is usually located on substrata composed of sand and dead coral rubble. *H. atra* specimens divide into two parts (fission) at a point located about 45 per cent of the length of the body from the mouth (Conand & de Ridder, 1990; Conand 1996). Each part then regenerates, thereby giving rise to two new identical specimens. This phenomenon affects a significant percentage of the specimens of the population at the Planch'Alizés study site (between 11.4 and 35 per cent according to Boyer, Caillasson & Mairesse 1995; Conand 1996).

This study was conducted through biannual samplings in a 80 m^2 section marked out by permanent plot markers. The goal of the study was to monitor changes in the population over a period of four years (November 1993 to November 1997), for a variety of parameters (fission and regeneration rates, population density, specimen size), in order to determine the significance and effects of asexual reproduction through fission on this population.

There were two broad categories of specimens: normal specimens and those involved in asexual reproduction, which made it possible to classify specimens according to six different categories (Doty, 1977; Conand & de Ridder, 1990; Conand, 1996):

- 'N' (normal) specimens: showed no signs of asexual reproduction;
- 'F'(fission) specimens: showed signs of transverse division (i.e. constriction at a point 45% of the length of the body from anterior section);
- 'A' (anterior) specimens: had recently undergone fission and only had their anterior part;
- 'P' (posterior) specimens: had recently undergone fission and only had their posterior part;
- 'Ap' (Anterior–posterior) specimens: showed signs of regenerating their posterior part;
- 'Pa' (Posterior–anterior) specimens: showed signs of regenerating their anterior part;
- an 'S' category: included all specimens resulting from fission (F, A, P, Ap, Pa).

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In order to show the influence asexual reproduction had on this population we monitored:

- fission (S(%)=[(A+P)/2T]*100) and regeneration (R(%)=[(Ap+Pa)/2T]*100) rates, according to the work begun by Boyer et al., 1995 and Conand, 1996. (A+P) corresponded to specimens which had recently undergone fission, (Ap+Pa) corresponded to specimens in the process of regenerating and (T) to the total number of specimens sampled;

- the seasonal nature of asexual reproduction, by comparing samplings corresponding respectively to summer (November) and winter (June) for those specimens which had recently undergone asexual reproduction (A+P) and those in the process of regeneration (Ap+Pa);

- changes in population density, using a variance test to determine if there were any population fluctuations over time;

- changes in weight distribution over the four-year period for the normal specimen (N) category in order to observe the influence of fission on specimen size over the medium term. To do so, various modal weights were determined by graphic analysis of weight distributions according to the modal progression analysis method.

Results

Percentages of the various categories of specimens sampled as well as averages and standard deviations are been given in Table 1.

Fission rates, regeneration rates, seasonal nature of fission

The results on fission rates S (%) obtained from the data in Table 1 are given in Figure 1 (see page 14).

The average fission rate was 3.7 per cent and the standard deviation equalled 2.1. Fission rates calculated in this manner showed two distinct periods on the histogram (Figure 1). The first period was from November 1993 to June 1995, during which time the rate was high (>3.7%) and a second period extending from November 1995 to November 1997, during which time the rate was lower (< 3.7%). Although the June 1996 rate (1.3%) was the lowest of all those recorded, it can be seen that the average rate for the month of June (4.3%) was higher than the average rate for November (3.3%).

Moreover, it was observed that after a sharp decrease in June 1996, the rate again increased in the following reading. It can be surmised that something happened during this period which had an influence on fission.

Table 1: **General data on the number of specimens, various percentages, averages and standard deviations for normal specimens (N), those which had recently undergone fission (A+P), those in the process of regenerating (Ap+Pa), those in fission (F), total specimens (T) and all specimens involved in asexual reproduction (S).**

Dates	T	N	A+P	Ap+Pa	F	S
Nov-93	293	219	37	32	1	74
		74,7%	*12,6%*	*10,9%*	*0,3%*	*25,3%*
Jun-94	362	276	50	23	13	86
		76,2%	*13,8%*	*6,4%*	*3,6%*	*23,8%*
Nov-94	387	303	25	59	0	84
		78,3%	*6,5%*	*15,2%*	*0,0%*	*21,7%*
Jun-95	433	372	41	19	1	61
		85,9%	*9,5%*	*4,4%*	*0,2%*	*14,1%*
Nov-95	421	369	18	34	0	52
		87,6%	*4,3%*	*8,1%*	*0,0%*	*12,4%*
Jun-96	393	373	10	10	0	20
		94,9%	*2,5%*	*2,5%*	*0,0%*	*5,1%*
Nov-96	390	358	15	17	0	32
		91,8%	*3,8%*	*4,4%*	*0,0%*	*8,2%*
Nov-97	376	326	21	28	1	50
		86,7%	*5,6%*	*7,4%*	*0,3%*	*13,3%*
Total	3,055	2596	217	222	16	459
		85,0%	*7,1%*	*7,3%*	*0,5%*	*15,0%*
Mean	381,9	324,5	27,1	27,8	2,0	57,4
Standard deviation	42,6	55,6	14,0	14,9	4,5	23,7

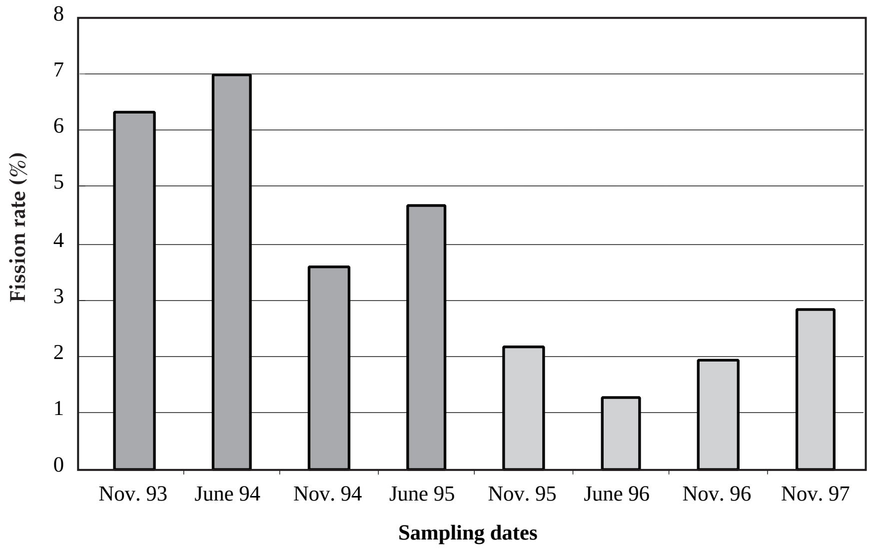

Figure 1: **Variations in the fission rate between November 1993 and November 1997**

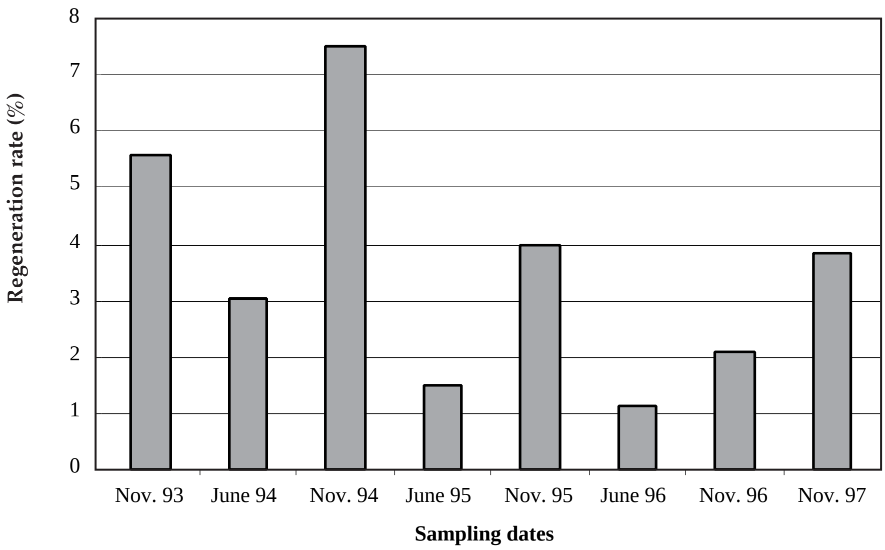

Figure 2: **Variations in the regeneration rate between November 1993 and November 1997**

Regeneration rates R (%), were calculated using the same formula as for fission rates, by simply replacing (A + P) specimens (in fission) by (Ap + Pa) specimens (those in regeneration), (Figure 2).

The average regeneration rate was 3.7 per cent. The average regeneration rate for the month of November (4.6%) was double the average rate for June (2.2%). These results were the opposite of those obtained for fission. As with fission rates, the June 1996 figure (1.2%)was the lowest of all.

Comparison of average fission and regeneration rates revealed equal values of 3.7 per cent for both, which led us to suppose that fission and regeneration periods were equal in length, and that the mortality rate was almost nil.

The dates selected for sampling (November – June) allowed us to study how significant asexual reproduction was in this population.

Readings for the warm (November) and cool (June) seasons did, in fact, alternate, except in 1993 (beginning of the study in November 93) and 1997 (no reading for June 1997).

Analysis of the changes in density for those specimens which had recently undergone fission and those in the process of regeneration (Figure 3) showed that the two curves (i.e. changes in the density of specimens which had recently undergone fission (A+P) and specimens in the process of regeneration (Ap + Pa)) alternated between high values and lower ones.

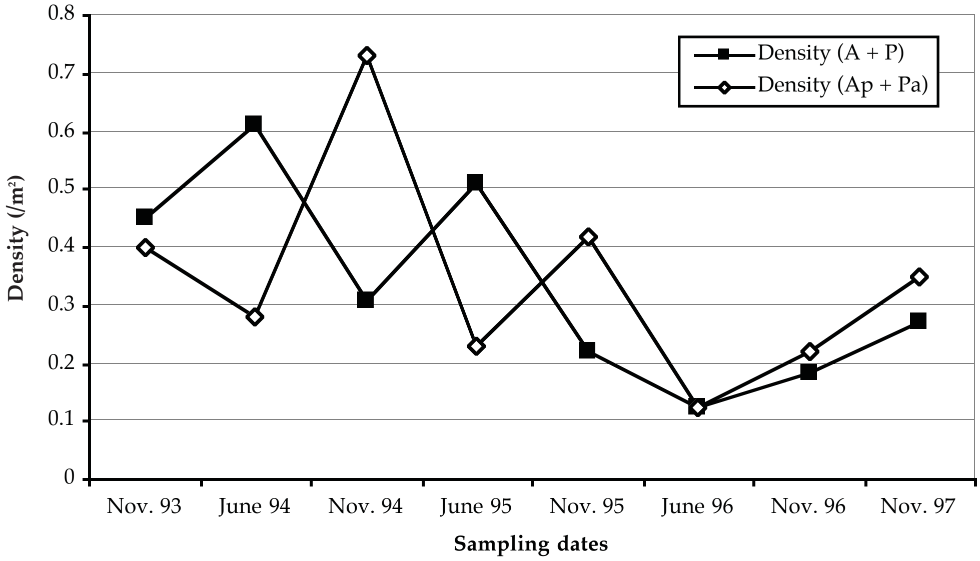

Figure 3: **Changes in density, between November 1993 and November 1997, for those specimens which had recently undergone fission and those in the process of regeneration**

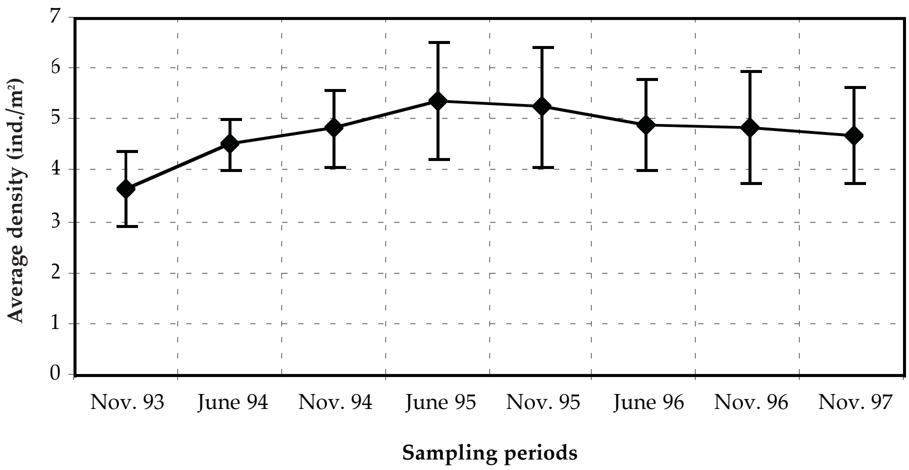

Figure 4: **Changes in the average number of specimens (± standard deviation) per sample (November 1993 – November 1997).**

Density among specimens in the process of fission (A+P) was lower in the month of November (in 1993: 0.45; in 1994: 0.30; in 1995: 0.20) than in June (in 1993: 0.65; in 1994: 0.50). Density among specimens in the process of regeneration showed an inverse tendency, i.e. lower values in the month of June (June 1994: 0.30; November 1994: 0.75; June 1995: 0.20; November 1995: 0.40).

This led us to suppose that the regeneration took about six months. This six-month period is shown on Figure 3 by the intervals between the successive peaks and troughs. For both curves, the figures obtained in readings in June 1996 were the lowest of all those taken (A+P:0.10; Ap+Pa: 0.10).

Finally, analysis of the final three readings show a very large difference in relation to the overall shape of the first part of the curve, due to the large decrease in density among both categories of specimens studied in June 1996. Comparison of the averages from June and November for the number of specimens resulting from recent fission (A+P) confirmed that averages for June were significantly higher, by almost one per cent, than averages for November.

Changes in specimen density and weight

Figure 4 shows changes in the *H. atra* population over time for all eight quadrats (80 m^2) sampled.

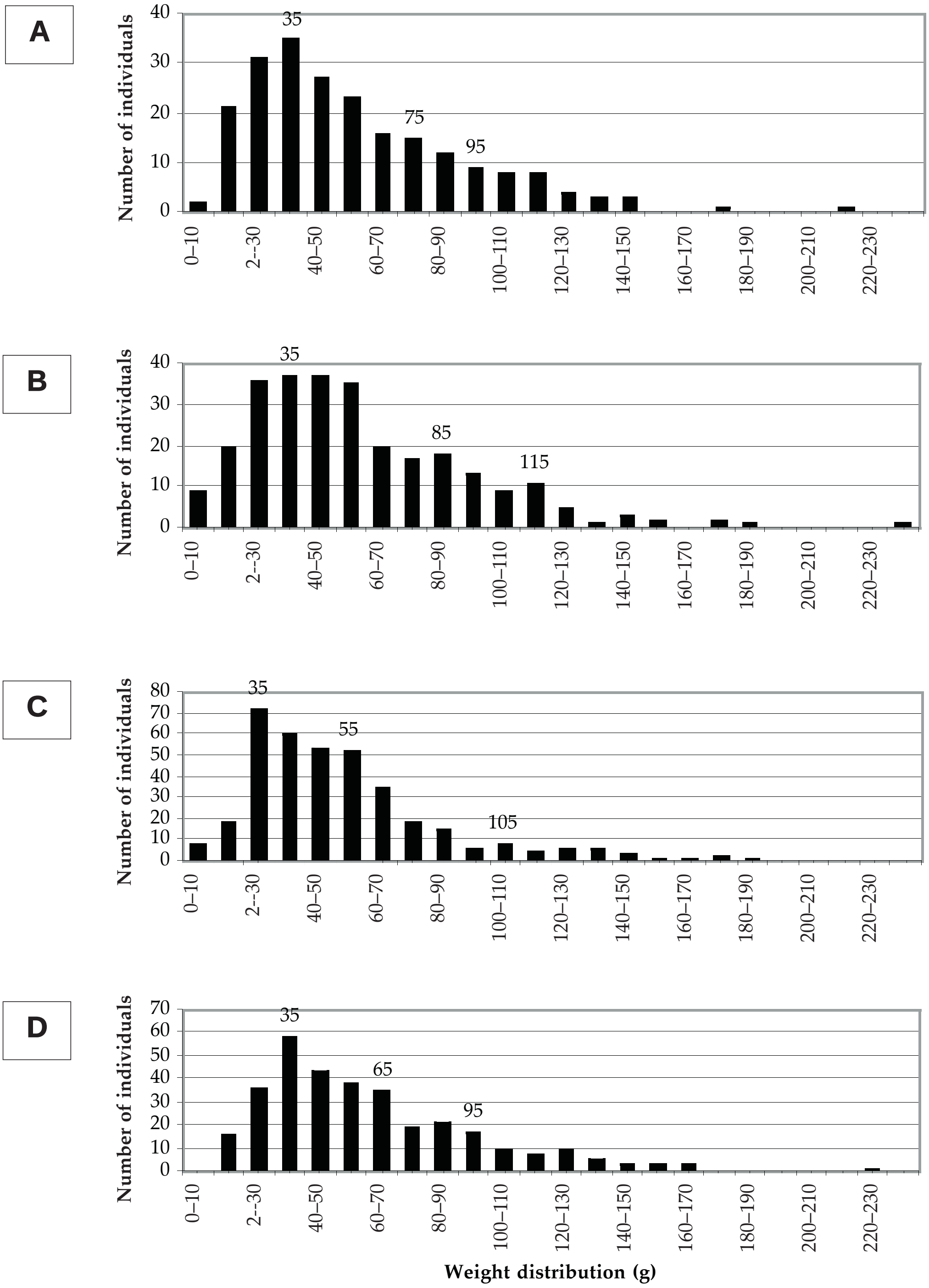

Figure 5 (A–D): **Changes in modal weight values in the weight distribution of normal specimens for samples taken in November 1993 (A) and 1997 (D) and June 1994 (B) and 95 (C).**

Overall, the curve remained relatively stable for the average number of specimens in this sea-cucumber population. However, two opposing trends were revealed: during the first part of the study (November 1993 – June 1995), the population increased in a slow but steady fashion; after June 1995, it decreased very slightly up to the month of November 1997.

The weight distribution for normal specimens (N) was plurimodal, but only the first three modal weights were taken into consideration, as the number of specimens in weight categories over 130 g was too low. These modal weights are shown in Figure 5, so that the changes in them over the four-year study period can be observed.

Weight distributions of normal specimens given in Figure 5 are based on sampling from November 1993 and 1997 and June 1994 and 1995. The first mode for all samplings combined (Nov. 93 to Nov. 97) varied from 15 to 35 g. The second mode calculated varied from 35 to 85 g and the third from 75 to 115 g. It can be observed that the values for the first mode were very similar in both November 1993 and November 1997 (35 g). The difference between the highest and lowest values of the first mode was 20 g, for the second 40 g and for the third 50 g. These differences indicate variations in specimen weights for certain years, but study of all samplings as a group did not show any changes in terms of either an increase or decrease in specimen weight for this population, since the figures at the beginning (Nov. 1993) and end (Nov. 1997) of the study were very similar, i.e. respectively, 35 g, 75 g, 95 g in November 1993 and 35 g, 65 g, 95 g in November 1997.

Discussion

Fission rates, regeneration rates and seasonal nature of fission

The fission rate S (%) during the study period (Nov. 1993 to Nov. 1997), calculated from those specimens that had recently undergone fission (A+P), was 3.7 per cent. It was lower than that calculated by Conand (1996), i.e. 4.5 per cent over the period of one year (1994), using monthly samplings. These differences can be explained in part by the extremely low fission rate observed in June 1996 (1.3%). The regeneration rate R (%) for *H. atra* during this study was 3.7 per cent. It would seem, then, that *H. atra* had a mortality rate of almost zero (S(%)=R(%)) for specimens in the process of regeneration. If there was almost no mortality in specimens that had undergone fission, the percentage of clones in the population should be increasing, since specimens resulting from fission can in turn give rise to two identical individuals. Over the long term this could lead to a drop in genetic diversity, which could have serious consequences for the conservation of this species.

The seasonal nature of the alternating high and low values for this rate, both in specimens that have recently undergone fission (A+P) and in those in the process of regeneration (Ap+Pa), was demonstrated. The density of (A+P) specimens was higher (test of comparison of mean significant at 1%) in June (cool season) than in November (warm season). Similar results were observed for this species over shorter study periods in New Caledonia (Conand & de Ridder, 1990), Taiwan (Chao et al., 1994) and on Great Palm Island, GBR (Uthicke, 1997). Boham and Held (1963) suggested that fission in *H. atra* may be caused by variations in water temperature and Conand (1989) thought that emersion time in New Caledonia may play an important role in fission.

Given both the fact that the area studied was located on the back reef and Reunion Island's tide characteristics, it can be deduced that there is never any emersion there. Moreover, the water temperature curve at the station for the period around June 1996 did not indicate anything abnormal in relation to average temperatures for that season. The low June 1996 rate seemed, then, to have been caused by other factors. Water salinity during these periods was also studied, but the readings did not show any anomalies. Uthicke (1997) theorised that the higher oxygen rate of the water during the cool season could facilitate sea cucumbers' regeneration after the total loss of the respiratory tree in *H. atra*. If eutrophisation of the environment occurred in June 1996, this could explain the sharp drop in the fission rate observed in Figure 3. The theory of eutrophisation of the environment was also raised by Conand et al., 1997 for a *H. leucospilota* population located on the back reef of La Saline, which, like our study site, is an area subject to significant human activity.

Specimen density and weight trends over time

Average densities in the quadrats were between 3.1 and 6.35 specimens per m^2. The overall average in this sector was 4.8 specimens per m^2 over a period of four years, which is relatively close to the figure calculated by Conand (1996) for the same population during the period 1993–1994. This average density figure is about 40 times greater than that observed for the same species on Rib Reef and about 10 times greater than the density of *H. atra* on Fantome Island (Uthicke, 1997). In contrast to Uthicke's results (1997), which noted higher density levels in populations that had a high fission rate (Rib Reef : S(%) = 9%, density: 10 specimens per 100 m^2; Fantome Island: S(%) = 76 per cent, density: 42 specimens per 100 m^2), at Planch'Alizés, despite very high density (4.8 specimens per m^2), the fission rate was only 3.7 per cent. The population seemed to have achieved optimum density in relation to the biotic and abiotic conditions of the environment (i.e. small back reef where only limited amounts of food are available). This would explain the low fission rate, which only allowed the species to maintain optimum density (Harriott, 1982; Conand & de Ridder, 1990; Chao et al.; 1993a; Chao et al., 1994; Uthicke, 1997). This theory could explain the low fission rate in 1996, which, perhaps, was not an 'accident' but rather the result of the high densities observed in June and November 1995. These may have been higher than the optimum density (Chao et al., 1994) in these environmental conditions for this species (density< 5 specimens per m^2). Overall, over the period of four years, asexual reproduction did not bring about any increase in population density.

As for weight trends over time, while there were significant differences in modal weight values over the course of a single year, in relation to the overall study they remained relatively stable. Since weights were constant and so were average sizes, asexual reproduction did not bring about any decrease in the overall size and weight of normal *H. atra* specimens. It is difficult to find any correlation between the size and age of specimens, as there is no clear correlation between the various modal weights. In fact, determining possible age-groups was not simple, due to the influence of asexual reproduction. After regeneration, the weight of normal specimens can be low and so directly influence modal values, since during regeneration specimens stop eating but increase the amount of energy they spend and this has a negative influence on their growth rate (Chao et al., 1994). Futhermore, variations in modal weight within the weight distribution of two successive samplings can be explained by the availability of food and the influence of other factors modifying environmental conditions (Chao et al., 1994).

In conclusion, asexual reproduction in *H. atra* on Reunion Island was seasonal in nature, with a higher fission rate during the cool season. Regeneration took about five to six months. Population density and average specimen weight remained relatively stable throughout the study (four years), which could confirm the theory that asexual reproduction allows a 'threshold-density' regulated by environmental conditions and the availability of food to be maintained.

A more detailed study of environmental factors would allow a better understanding of the extrinsic causes of fission, as endocrinal mechanisms could also exist.

References

BOYER, C., S. CAILLASSON & K. MAIRESSE (1995). Asexual reproduction in *Holothuria atra* on a reef of Reunion Island in the Indian Ocean. SPC Beche-De-Mer Information Bulletin 7: 7–9.

CHAO, S.-M., K.-H. CHANG (1989). The shallow-water holothurians (Echinodermata: Holothuridea) of southern Taiwan. Bull. Inst. Zool., Acad. Sin. 28: 107–137.

CHAO, S.-M., C.-P. CHEN & P.S. ALEXANDER (1994). Reproduction and growth of *Holothuria atra* (Echinodermata: Holothuridea) at two contrasting sites in southern Taiwan. Mar. Biol. 119: 565–570.

CONAND, C. (1989). Les holothuries aspidochirotes du lagon de Nouvelle-Calédonie: biologie, écologie et exploitation. Études et théses. ORSTOM, Paris, 393 p.

CONAND, C. (1996). Asexual reproduction by fission in *Holothuria atra*: Variability of some parameters in population from the tropical Indo-Pacific. Oceanologica acta 19,3: 209–216.

CONAND, C. & C. DE RIDDER (1990). Reproduction asexuée par scission *chez Holothuria atra* (Holothuridea) dans des populations de platiers récifaux: 71–76. **In:** Echinoderm Research. de Ridder et al. (Eds). Balkema, Rotterdam: 343p.

CONAND, C., C. MOREL & R. MUSSARD (1997). A new case of asexual reproduction in holothurians: fission in *Holothuria leucospilota* populations on La Reunion Island in the Indian Ocean. SPC Beche-de-Mer Information Bulletin 9: 5–11.

DOTY, J.E. (1977). Fission in *Holothuria atra* and holothurian population growth. M. Sc. Thesis, University of Guam: 54p.

HARRIOT, V.J. (1982). Sexual and asexual reproduction of *Holothuria atra* Jaeger at Heron Island Reef, Great Barrier Reef. Australian Museum Memoir, 16: 53–66.

UTHICKE, S. (1994). Distribution patterns and growth of two reef flat holothurian *Holothuria atra* and *Stichopus chloronotus*. **In:** David D., Guille A., Feral J.P., Roux M. (eds) Echinoderms through time. Proceeding of the Eighth International Echinoderm Conference. A.A. Balkema, Rotterdam.

UTHICKE, S. (1997). The seasonnality of asexual reproduction in *Holothuria atra*, *Holothuria eludis* and *Stichopus chloronotus*. (Holothuridea: Aspidochirotida) on the Great Barrier Reef. Mar. Biol. 129: 435–441.