

Fission in a population of *Stichopus chloronotus* on Reunion Island, Indian Ocean

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Introduction

Although *Stichopus chloronotus* is found throughout the tropical Indo-Pacific region, this species has not yet been studied in depth. In New Caledonia, it is mainly found on outer reef flats and upper zones of outer slopes at depths of less than 12 m (Conand, 1989). On the Australian Great Barrier Reef, it has a similar distribution, with higher densities on the outer reef flat than on the inner reef flat (Franklin, 1980). It only feeds between 11 a.m. and 7 p.m., and at night hides under dead coral or large debris (Uthicke, 1994).

As for sexual reproduction, two spawning periods have been observed in Australia, in November and March (Franklin, 1980). Asexual reproduction through fission was observed for the first time in Australia by Harriot (1980) and Franklin (1980) and the seasonal nature and the extent of this event were recently studied by Uthicke (1997).

This study conducted in La Réunion presents fission and regeneration rates for a main population of *Stichopus chloronotus* as well as the morphology and anatomy of specimens in the process of regeneration. The results are compared to those for the population of another reef where fission was not observed. These two strategies are linked to population density and specimen size.

Materials and methods

Observations were carried out in October 1996 and in April and July 1997 at the main 'Trou d'eau' station, located on the Saint Gilles/La Saline fringing reef on the west coast of the island. The back reef, where the population was located, forms a channel and consists mainly of deposits of biological detritus and an accumulation of dead coral, coral sand and basalt boulders; sea urchins (*Echinometra mathaei*, *Stomopneustes variolaris*, *Diadema setosum*) and cowry shells were also observed there. The *Stichopus* were observed grouped together on coral

heads; the quadrats where there were no specimens consisted primarily of sand with very little coral.

During each observation, data were collected in 20 marked quadrats, each one square metre in size. All the specimens of *Stichopus chloronotus* found were collected. This count allowed assessment of distribution and average density for the species at this site, comparison of these parameters to those observed elsewhere and assessment of fission and regeneration rates.

The various categories of specimens were defined as had already been done for other species (Conand & De Ridder, 1990; Conand et al., 1997). In the case of *Stichopus chloronotus*, only three categories of specimen were observed with any frequency, these being normal specimens (N) and two types of specimen in the process of regenerating (Ap) and (Pa). Ap are those specimens corresponding to the anterior part after fission which are regenerating their posterior part. Pa specimens are those corresponding to the posterior part after fission which are regenerating their anterior part.

The first task carried out in each quadrat was to identify the various categories of specimens. Measurements of the total length of each specimen were taken using a measuring tape; the regenerated part was also measured and specimens were weighed.

Several specimens of each category were then collected at the 'Trou d'eau' site for dissection. Measurements were taken at two separate stages:

- on fresh animals after relaxation using magnesium chloride:
 - Lt:** total length, ± 0.5 cm along the back from mouth to anus;
 - Lr:** length of the regenerated part, ± 0.1 cm;
 - Wt:** total weight of the specimen, ± 0.5 g; and
 - Wd:** drained weight after the coelomic liquid had been drained off, ± 0.5 g;

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- on specimens preserved in Bouin:
 - Li:** length of the digestive tube, ± 0.5 cm;
 - Lg:** length of the gonads, ± 0.5 cm;
 - Wif:** weight of the full digestive tube, ± 0.5 g;
 - Wie:** weight of the empty digestive tube, ± 0.5 g;
 - Wg:** weight of the gonads, ± 0.1 g;
 - We:** weight of the tegument, ± 0.5 g;

These measurements made it possible to calculate both digestive tube contents: $Wi\ f-e = Wif - Wie$, and gonad-index: $RGS = Wg (Pg) * 100 / We$.

At the supplementary 'Étang Salé' station, a fringing reef located further to the south, sampling conducted in February 1997 made it possible to calculate the density and structure (in weight) of a second population. No specimens in the process of fission or regeneration were observed there.

Results

Presentation of the two populations studied

At the 'Trou d'eau' station, the population was composed of a mixture of normal specimens and specimens in the process of regenerating after fission.

Table 1 presents the figures and percentages for each category on the three sampling dates. The average density was 3.7 individuals per square metre (m^2). On average, normal specimens made up 83 per cent of the sample, while specimens in the process of regeneration were divided between 9 per cent for those which were regenerating their anterior part and 7 per cent for those regenerating their posterior part. Specimens in the process of regeneration were more numerous in October (24%) than in April (11%) or July (13%).

Figure 1 shows the weight distribution for both normal specimens and those in the process of regeneration at this station on the three observation dates. Normal specimens weighed up to 140 g and the weight distributions showed two modes, one at 25 g and the other at 65 g, whereas specimens in the process of regeneration had only one modal weight at 15 g.

At 'Étang Salé', the population consisted only of normal specimens, which were quite large in size (Figure 2), that is, between 55 and 265 g. The density there was low, at 0.17 per m^2 .

Comparative anatomy of the various specimen categories

Comparison between normal specimens and the two categories of specimens in the process of regeneration led to a better understanding of the dynamics of regenerating the main organs. It was then possible to infer when feeding and reproduction (in particular) began again after fission.

Observation of eight normal specimens (Table 2 and Figure 3 B, see next pages) showed that at the 'Trou d'eau' study site, *Stichopus chloronotus* had an average length of 17.3 cm, and an average overall open weight of 66.3 g.

The tegument of *Stichopus chloronotus* is green, thick, smooth and has a tendency to disintegrate rapidly. The weight of the tegument (muscle included) (We) averaged 39.6 g, and it was 3 mm thick. The muscles of normal specimens were characterised by five longitudinal muscular bands that were attached from the peripharyngeal calcareous ring up to the cloaca. Each muscular band had an

Table 1 : Distribution of the various categories of specimen at 'Trou d'eau' for the three sampling dates

Date	Total no.	Normal	A + Ap	P + Pa	F	S
30.10.1996	78	59	9	10	0	19
01.04.1997	53	47	1	5	0	6
15.07.1997	93	81	6	6	0	12
Total	224	187	16	21	0	37
% of total		83 %	7 %	9 %	0 %	17 %
Mean	74.7	62.3	5.3	7.0	0.0	12.3
S. d.	20.2	17.2	4.0	2.6	0.0	6.5

F: Specimens in the process of fission; S: Specimens having completed fission (A + Ap + P + Pa)

average width of 5.5 mm and was attached to the tegument in its median part.

Stichopus chloronotus is a detritus-feeder species which uses its twenty oral tentacles (averaging 4.8 mm in length) to collect food. The digestive tract had a thin wall and formed three loops. The average length of the digestive tract was 302.4 mm, with an empty weight of 2.3 g.

Using these data, it was possible to estimate the amount of food consumed by specimens at the

time of harvest. This varied between 0.4 g and 9.0 g with an average of 4.6 g. The transverse vessel linked the first loop to the second. The *rete mirabile* was located on the second loop.

Water vascular system: the turgescence of the oral tentacles comes from the 20 vesicles of the oral podia situated in pairs around the peripharyngeal calcareous ring. The average length of the vesicles of the oral podia was 22.4 mm. The Polian vesicle, situated under the peripharyngeal calcareous ring, measured 15 mm.

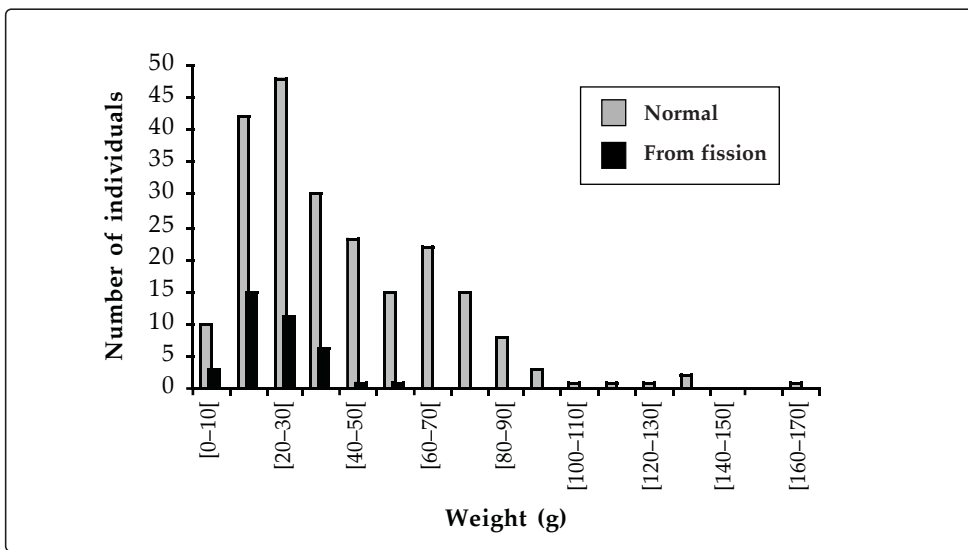


Figure 1
Weight distribution of normal specimens and those resulting from fission at 'Trou d'eau'

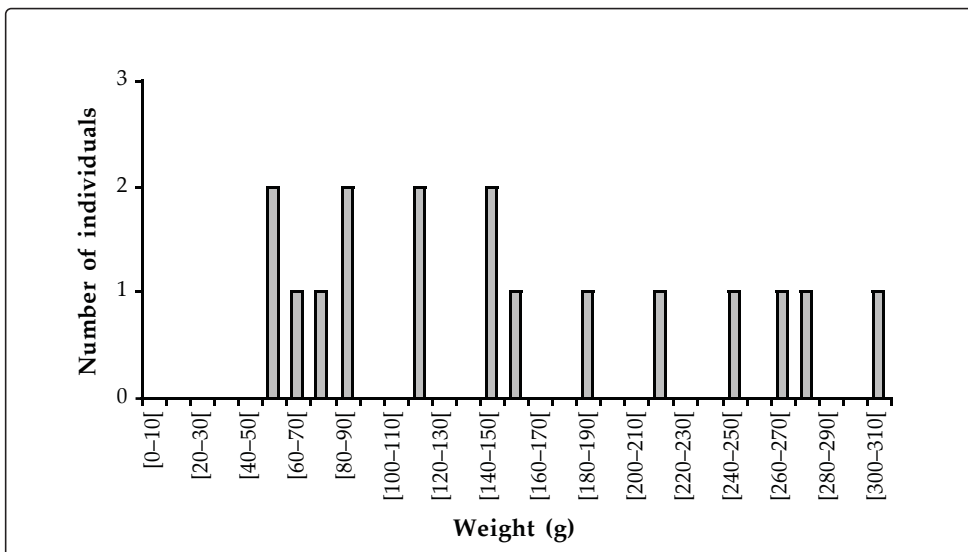


Figure 2
Weight distribution of specimens at 'Étang Salé'

Table 2: Parameters of normal individuals (N)¹

	Wt (g)	Wd (g)	We (g)	Lt (mm)	Wi f (g)	Wi e (g)	Wi f-e (g)	Li (mm)	Wg (g)	Lg (mm)	RGS
N 20	94.60	85.50	53.88	18.00	6.38	3.30	3.08	343.00	0.70	114.00	1.30
N 21	63.80	55.50	39.69	14.20	-	-	-	237.00	0.11	26.00	0.28
N 22	92.00	89.60	42.25	18.40	11.70	2.69	9.01	310.00	0.93	120.00	2.20
N 23	85.00	83.20	53.08	17.70	10.16	2.37	7.79	299.00	0.56	126.00	1.06
N 24	66.10	63.60	32.66	16.00	9.67	1.96	7.71	323.00	0.21	4.50	0.64
N 26	55.20	53.70	31.70	18.00	2.36	1.97	0.39	250.00	0.17	112.00	0.54
N 27	43.30	41.30	27.10	17.50	3.30	1.48	1.82	230.00	0.02	34.00	0.07
N 28	60.30	58.10	36.36	18.50	4.77	2.23	2.54	370.00	0.03	84.00	0.08
Mean	70.04	66.31	39.59	17.29	6.91	2.29	4.62	295.25	0.34	77.56	0.77
S. d.	17.30	16.48	9.15	1.38	3.37	0.54	3.19	48.18	0.32	45.55	0.68
Var.	299.23	271.51	83.75	1.89	11.39	0.29	10.19	2320.94	0.10	2074.59	0.46

Table 3: Parameters of anterior individuals regenerating the posterior (Ap)¹

	Wt (g)	Wd (g)	We (g)	Lt (cm)	Wi f (g)	Wi e (g)	Wi f-e (g)	Li (mm)	Wg (g)	Lg (mm)	RGS	Lr (cm)
Ap 1	51.00	48.00	28.27	-	0.79	0.47	0.32	143.50	0.03	20.00	0.11	1.50
Ap 3	42.60	36.60	22.82	12.50	0.98	0.40	0.58	128.00	0.04	33.00	0.18	2.50
Ap 4	40.00	33.00	21.99	11.50	0.80	-	-	174.50	0.04	2.20	0.18	1.50
Ap 7	26.60	23.50	15.74	9.00	0.17	-	-	127.00	0.02	21.00	0.13	1.20
Ap 11	18.80	18.10	11.06	7.80	0.56	0.37	0.19	122.50	0.02	11.00	0.18	1.10
Ap 12	28.30	24.80	14.02	9.80	-	-	-	160.00	0.02	19.00	0.14	1.80
Ap 15	5.00	4.90	2.98	4.50	0.18	0.15	0.03	67.00	-	-	-	1.40
Ap 16	13.70	12.60	7.88	8.10	0.29	0.27	0.02	172.00	0.03	18.00	0.38	1.80
Ap 17	14.30	12.40	7.68	7.40	0.33	0.18	0.15	129.00	0.02	5.00	0.26	0.30
Mean	26.70	23.77	14.72	8.83	0.51	0.31	0.22	135.94	0.03	16.15	0.19	1.46
S. d.	14.45	12.82	7.83	2.34	0.29	0.12	0.19	30.82	0.01	9.21	0.08	0.56
Var.	208.76	164.48	61.24	5.49	0.09	0.01	0.04	949.75	0.00	84.91	0.01	0.32

Table 4: Parameters of posterior individuals regenerating the anterior (Pa)¹

	Wt (g)	Wd (g)	We (g)	Lt (cm)	Wi f (g)	Wi e (g)	Wi f-e (g)	Li (mm)	Wg (g)	Lg (mm)	RGS	Lr (cm)
Pa 2	30.10	24.20	13.16	9.80	0.12	0.11	0.01	89.00	0.01	3.00	0.08	1.10
Pa 8	22.80	18.60	11.49	8.50	0.08	0.07	0.01	86.00	0.01	7.00	0.09	1.20
Pa 9	15.60	12.20	8.49	7.50	0.06	-	-	59.00	-	-	-	1.30
Pa 10	21.60	19.80	12.62	7.80	0.11	0.10	0.01	96.00	-	-	-	0.40
Pa 13	13.00	12.50	8.34	7.60	0.16	0.16	0.00	122.00	-	-	-	1.60
Pa 14	8.50	8.40	5.69	6.30	0.16	0.16	0.00	101.00	-	-	-	1.10
Pa 18	19.40	11.80	7.86	8.30	-	-	-	118.00	-	-	-	1.40
Mean	18.71	15.36	9.66	7.97	0.12	0.12	0.01	95.86	0.01	5.00	0.08	1.16
S. d.	6.57	5.18	2.58	0.99	0.04	0.04	0.00	19.65	0.00	2.00	0.01	0.35
Var.	49.12	26.81	6.64	0.99	0.00	0.00	0.00	386.12	0.00	4.00	0.00	0.12

1. Wt: total weight; Wd: drained weight; We: eviscerated weight; Lt: total length; Wi f: full intestine weight; Wi e: empty intestine weight; Wi f-e: intestine contents weight; Li: intestine length; Wg: gonad weight; Lg: gonad tubule length; RGS: gonad index; Lr: regenerated length

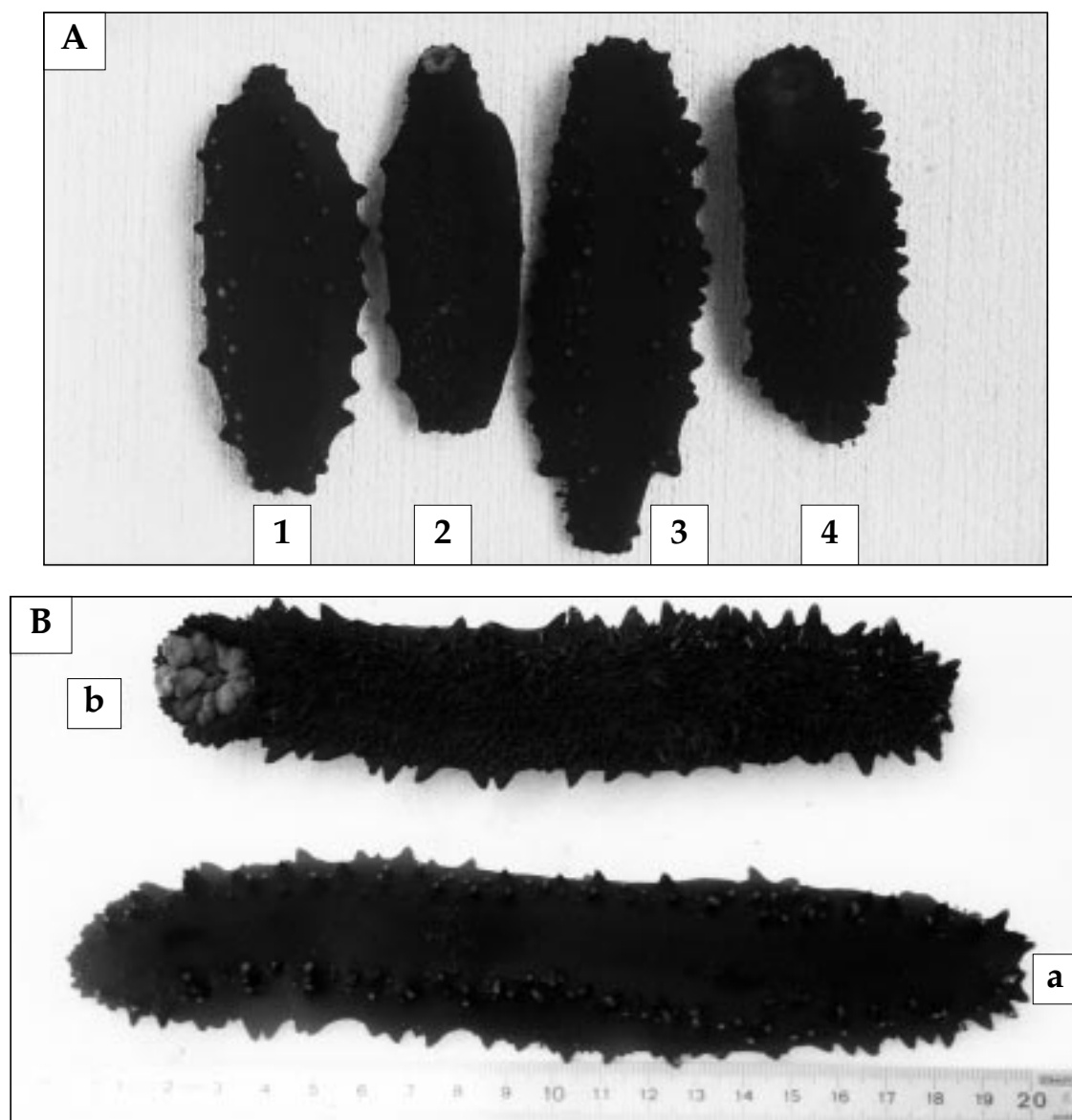


Figure 3

Stichopus chloronotus

Photo A: regenerating specimens: 1 & 2: Pa; 3 & 4: Ap.
Photo B: normal specimens: a: dorsal side; b: ventral side.

The genital glands were in the form of two tufts situated on either side of the dorsal mesentery, and were attached by the gonadal base. The gonads were in the form of branched tubules. The average weight of the gonads was 0.3 g. The gonad index was between 0.08 and 2.2. The average length of the gonads was 77.6 mm. No difference was observed between male and female gonads.

The respiratory system was formed from two branched tubes called respiratory trees, on the right and left, which opened into the cloaca and ex-

tended into the coelomic cavity. The left respiratory tree was intertwined with the *rete mirabile*, while the right respiratory tree was attached to the tegument by the mesentery.

Several specimens in the process of regenerating their anterior part (Pa) or posterior part (Ap) are shown in Figure 3 A.

Specimens in the process of regenerating their posterior part (Ap) are presented in Table 3 and Figure 4 (see next page).

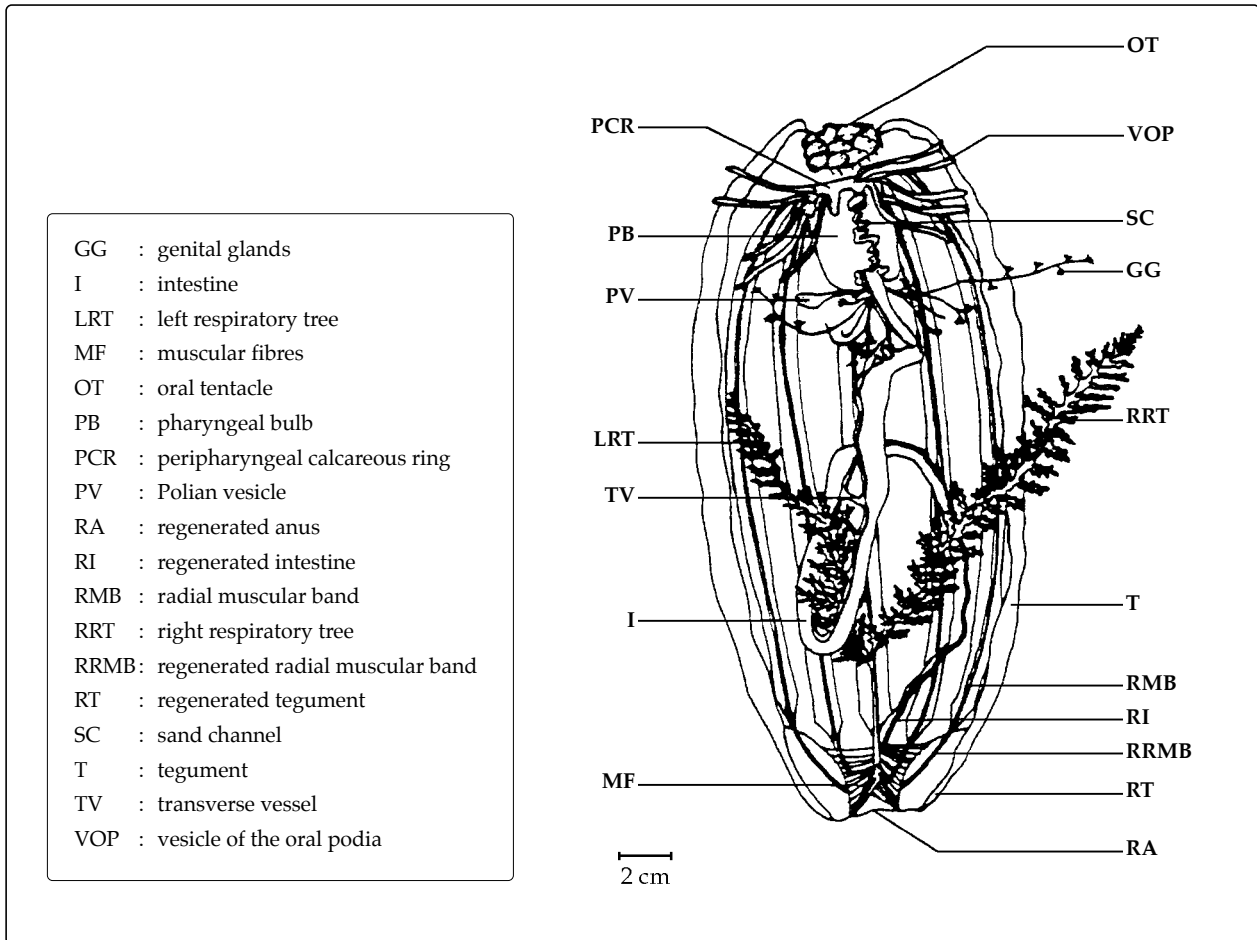


Figure 4

Anatomy of an Ap specimen

Ap specimens in the process of regeneration had an average length of 8.8 cm, or half that of normal specimens, and an average total open weight of 23.7 g. From an anatomical point of view, only the posterior part changed.

The regenerated tegument appeared lighter after fixation in Bouin. A fold separating the regenerated part from the anterior part was observed. The thickness of the tegument varied in three areas: the 'normal' part near the mouth measured 3 mm, the tegument near the fold measured 6 mm and the regenerated tegument measured 1 mm. The length of the regenerated tegument when retracted averaged 1.5 mm, while the original tegument averaged 7.4 cm. Moreover, a lack of papillae and a difference in the size of the tube feet between the regenerated part (0.5 to 1 mm) and the original part (2 mm) could be observed. The muscles also regenerated. In fact, the muscles in the anterior part had a width of 5.5 mm, while they only measured about 1.5 mm in the regenerated part. They were, then, thinner and they were attached to the tegument at the cloaca.

Ap specimens are characterised by regeneration of the posterior part of the digestive tract and the anus. The transverse vessel and an under-developed *rete mirabile* could be seen.

The average length of the digestive tract was 135.9 mm, with an empty weight of 0.3 g. According to data, the quantity of sand ingested into the digestive track of Ap individuals (0.2 g) was much less than in normal specimens. The presence of sand in the digestive track indicated that the specimen had sufficiently regenerated to allow normal feeding.

Gonads were present, but were not very developed. The gonadal base appeared to be barely formed. The average length of the genital glands was 16.1 mm, with a gonad-index varying between 0.11 and 0.38. It is thought that either these specimens had not reached sexual maturity or the gonads had regressed after fission.

In general, the respiratory trees observed in Ap specimens had a normal appearance. Fission seemed to have occurred behind these organs.

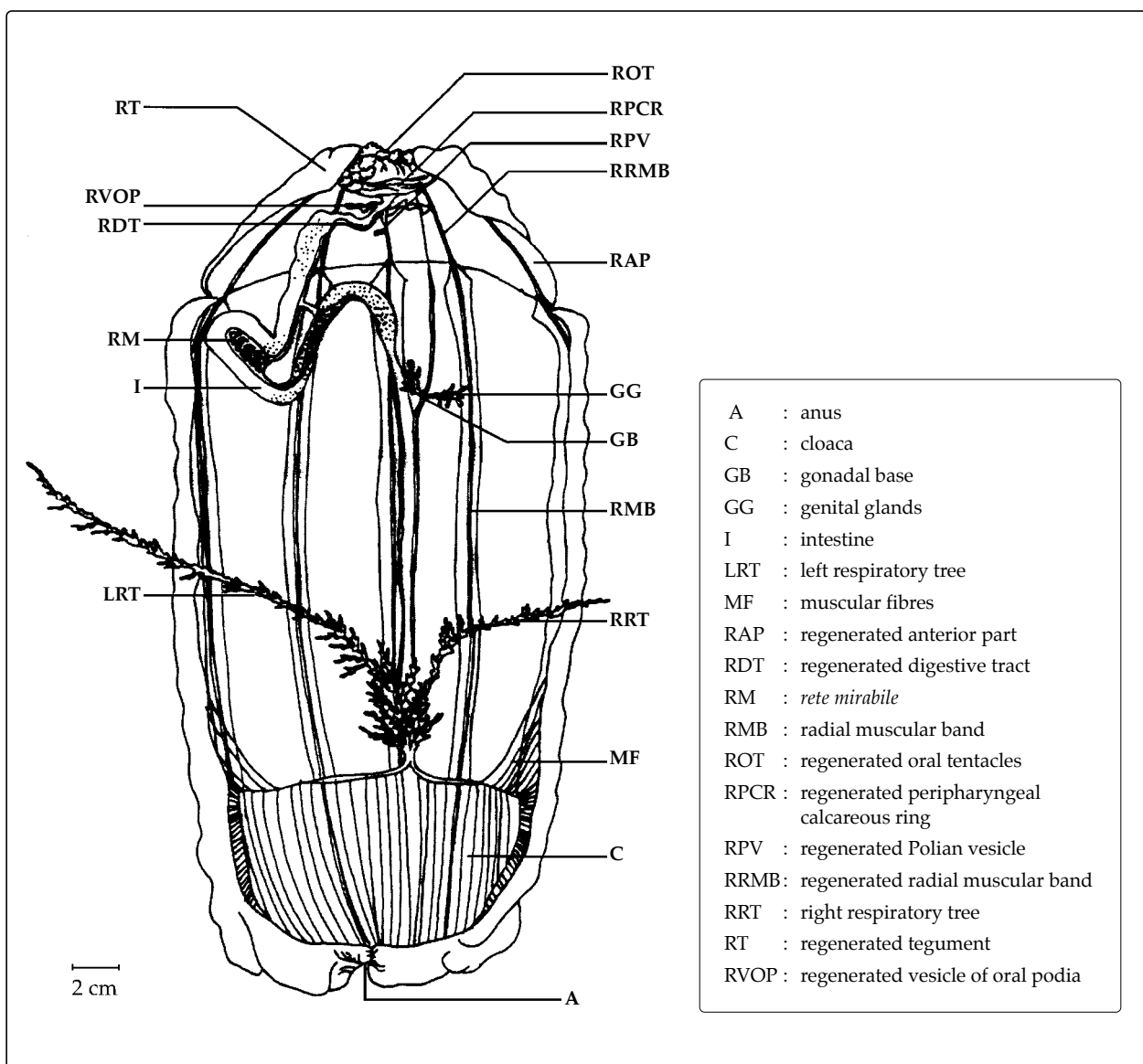


Figure 5
Anatomy of a Pa specimen

Specimens in the process of regenerating their anterior part (Pa) are shown in Table 4 (page 18) and Figure 5.

Pa specimens in the process of regeneration had an average length similar to that of Ap specimens, that is 8.0 cm; and they weighed between 8.5 and 30.1 g.

As with Ap specimens, the colour of the regenerated tegument was lighter. On average, the regenerated part measured 1.2 cm and had regenerated tube feet measuring 1.25 mm on its ventral surface but no papillae on the dorsal surface. The regenerated mouth had a diameter of 6.5 mm. The thickness of the tegument varied between 0.5 mm and 0.75 mm, and the muscles averaged 0.75 mm. A

folded area separated the original part from the regenerated one. The original part had an average length of 6.8 cm. The thickness of the tegument was 2.5 mm and the thickness of the muscles was 4.5 mm. On the ventral surface, tube feet measured about 2.5 mm.

The regeneration of Pa specimens involved various changes. The peripharyngeal calcareous ring was barely visible. The Polian vesicle had regenerated, but was small in size (about 6 mm). The three loops of the digestive track were formed and were very thin. The presence of the transverse vessel was noted in certain Pa specimens, and the *rete mirabile* was present but very under-developed. The amount of sand ingested by specimens

was almost nil. This small quantity, 0.01 g, leads to the supposition that the digestive track was not yet functional.

Depending on the stage of regeneration, the reproductive system varied; in two specimens, barely-formed gonadal bases were observed, while gonads were absent in five other specimens. The respiratory trees appeared more transparent than those of normal specimens, and were much less thick. None of the specimens had a left respiratory tree intertwined with the *rete mirabile*.

Discussion

Stichopus chloronotus is one of the most common sea cucumber species in La Réunion, together with *H. leucopsilota*, *H. atra* and *Synapta maculata*. Studies have already been carried out on *Stichopus chloronotus* in the South Pacific, but this is the first one to be conducted in La Réunion.

In La Réunion, population density reached 3.15 specimens per m² at the 'Trou d'eau' station. This figure is high in comparison with densities observed in New Caledonia, since they did not exceed 0.5 specimens per m² there (Conand, 1989), and those observed on the Great Barrier Reef, from 0.02 to 1.2 specimen per m² (Franklin, 1980; Uthicke, 1994 and 1997). Moreover, *S. chloronotus* was located on the back reef at 'Trou d'eau', while generally it is a species found on the outer reef flats, but the very narrow width of the reef may provide an explanation for this distribution.

According to Franklin (1980), sexual maturity occurs at the age of one year, when specimens reach a weight of 70 g. Gonads are whitish in colour in both sexes. The spawning period takes place in spring and in autumn (Franklin, 1980).

The average size of specimens varies according to the population studied, from 30 to 170 g on the Great Barrier Reef (Uthicke, 1994) and 50 and 150 g respectively for the two populations in La Réunion.

Despite the fact that the sample size in La Réunion was insufficient to demonstrate seasonal variations in fission rates, it would appear that, as on the Great Barrier Reef (Uthicke, 1997), fission was higher in July (cool season).

There was a marked contrast in La Réunion between the population of 'Trou d'Eau', with its high density, small specimens and high fission rate, and the 'Étang Salé' one, where fission was non-existent, the density low and the size relatively large. These results confirm those of Uthicke for the Great Barrier Reef (1997), where fission rates were correlated to population density.

The results obtained about fission and regeneration of this species are primarily related to frequency of regeneration rates.

During our study, no specimens in the process of fission were observed. The regeneration rate ($\%R = (Ap+Pa)/2T*200$) was 18.6 per cent for the population considered in October. Fission varied according to species; most of the time it was due to a constriction which led to a tear in the tegument, digestive tract and muscles. Fission occurred at about 52 per cent of the total length in this species, since Ap specimens were slightly larger than Pa ones. In *H. atra*, fission occurred at 45 per cent of the total length (Conand & De Ridder, 1990) and in *H. leucopsilota*, fission occurred at 22 per cent of the total length (Conand, Morel & Mussard, 1997).

As for weights, it was seen that for specimens in the process of regeneration, the weights Wt, Wd and We were about half those for normal specimens. This could be explained by the fact that Ap and Pa specimens do not feed during regeneration and by the lysis of the organs following fission until such time as the digestive organs again become functional.

In *Stichopus chloronotus*, the mortality of Ap specimens was slightly higher than that of Pa, as their numbers were lower. According to Doty's theory in 1997, mortality in Ap specimens is higher than in Pa ones in *H. atra*. According to Conand, Morel and Mussard (1997), mortality of specimens resulting from fission in *H. atra* is much higher than mortality of A and P specimens in *H. leucopsilota*.

Study of the anatomy of the two types of specimens undergoing regeneration, when compared to that of normal specimens, allowed a better understanding of the process of regeneration. The differentiation between the normal part and the regenerated part was marked on the external morphology by a small ring, which indicated a regenerated anus or mouth, narrower than the rest of the body. The tegument, as well as the width of the muscles and their attachments marked the area being regenerated and they converged towards the anus (Ap) or the mouth (Pa).

Generally, in Ap specimens, changes covered about a third of the specimens and were evident in the regenerated part only. In the specimens observed, the anus had already formed, and was strengthened to a certain degree by muscular fibres.

Pa specimens showed more changes than Ap specimens. In fact, in this case, it was the anterior part, or mouth which had to be regenerated. Depending on the stage observed, a peripharyngeal calcareous ring may have been observed, the

rete mirabile may have been absent or under-developed, and the transverse vessel may or may not have existed. The respiratory trees were present, but were not as developed as in normal specimens. One Pa specimen was observed with a respiratory tree that seemed to have been regenerated as it was much more transparent and thinner than the old one. The gonads were three times smaller than those of Ap specimens.

The anatomical study also allowed a better understanding of the resumption of feeding and reproduction in specimens resulting from fission.

Concerning feeding during regeneration, the quantity of material ingested averaged 0.22 g for Ap specimens and 0.01 g in Pa specimens. This indicated to us that digestive system activity was much more significant in Ap specimens. This could be explained by the fact that Ap specimens regenerate their anus and thus keep their buccal system intact. The average length of the digestive tract was greater in Ap specimens (135.94 cm) than in Pa specimens (95.86 cm). This ratio shows that the digestive tract of Ap specimens was on average 1.42 times greater than that of Pa specimens.

Finally, concerning reproduction, the gonads of Ap specimens were longer (16.15 mm) than those of Pa specimens (5 mm); but in comparison to normal specimens (78.10 mm), the average length of the gonads in the Ap specimens was 15 times smaller. It is supposed that the gonads break, leaving in place the gonadal base, which then regenerates. The gonad-index indicated that the gametes regenerate quicker in Ap specimens (0.19) than in Pa specimens (0.08).

Conclusion

This study on the asexual reproduction and regeneration of *Stichopus chloronotus* has made it possible for the anatomy of the species and the parameters for regeneration to be recorded in detail. This adds to the knowledge gained from the studies on *H. atra* and *H. leucospilota* in La Réunion, and indicates that fission is frequent there. A study under way on *H. atra* is aimed at quantifying the long-term consequences of this event on population dynamics, and to verify if it is determined by human disturbances which are particularly pronounced in La Réunion.

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