ticular sea cucumbers, is an objective to be achieved and that coordination and integration are necessary; meetings have taken place in the regions to better organise the sea cucumber trade by involving the various parties concerned (meetings were held at Ambanja, Mahajunga and Toliara). The Madagascar National Trepang Traders Group (ONET) was set up in Antananarivo on 25 September 1996.

The group's objectives are as follows:

- trepang quality management;
- training of traders and fishers in better resource management; and
- working closely with the administration in the management and exploitation of trepang.

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# A new study of asexual reproduction in holothurians: Fission in *Holothuria leucospilota* populations on Reunion Island in the Indian Ocean

*by C. Conand [1], C. Morel [1] & R. Mussard [1]*

## Introduction

*Holothuria leucospilota* is a large, black sea cucumber species, which is found throughout the tropical Indo-Pacific Region. It most often lives near the back reef, in sandy areas where dead coral accumulates (Massin & Doumenc, 1986; Conand, 1989; Ong Che, 1990). This initial study on Reunion Island proved that asexual reproduction through fission does exist, contrary to some previous observations (Britayev, 1992), and that it is even quite common.

The data collected covered information on the species populations and their densities, and on fission and regeneration rates in the different sites within the La Saline reef complex.

This data also allowed observations on the morphology and anatomy of normal individuals, those in fission and those in regeneration. Finally, the chronology of organ development during fission and regeneration was extrapolated from the data.

## Material and methods

All specimens of the species *Holothuria leucospilota* found within the 10 m$^2$ quadrats set out in the back reef of several sites in the Saint Gilles/La Saline Reef were collected, measured and weighted. The process of asexual reproduction in *H. leucospilota* leads to six distinct categories for specimens. The categories as defined for other species, in particular *Holothuria atra* (Conand & De Ridder, 1990; Conand, 1996), were used here for *H. leucospilota*.

- Normal specimens (N) which show no sign of fission.
- Specimens in the process of fission (F) are characterised by a constriction in the anterior part of the body.
- Complete fission of F specimens leads to two new types of specimen:

  (A) specimens which correspond to the anterior part,

  (P) specimens which correspond to the posterior part.
- A and P specimens will then regenerate into:

  Anterior specimens that are regenerating their posterior part are specimens (Ap).

  Posterior specimens that are regenerating their anterior part are specimens (Pa).

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Data on the frequency of occurrence for the different categories of specimens allowed fission figures to be determined (Conand, 1996):

The fission rate (F%) was calculated from A and P specimens using the formula (A+P)/2T*100, with T being the total number of specimens.

The regeneration rate (R%) was calculated from Ap and Pa specimens using the formula (Ap+Pa)/T*100.

The different types of specimens were collected and dissected in order to analyse the arrangement of internal organs during the various stages of fission and regeneration. Measurements were taken from specimens fixed with Bouin fluid, after relaxation using magnesium chloride.

The different length and weight measurements covered:

Total length, ± 0.5 cm, and the length of the regenerated part, ± 0.1 cm;

Length of the intestine and Polian vesicle, ± 0.5 cm; oral podia;

Total open weight and the weight of the integument, ± 0.5 g.

Weight of the intestine tube and gonads, ± 0.1 g;

The gonadal-somatic ratio was calculated, using the formula RGS=100*Pg/Pt, in order to determine the degree of sexual maturity.

## Results

### Abundance levels

The results first involved density levels, which averaged 0.84 'normal' specimens per sq.m. (variation: 0.09) and 0.12 specimens in regeneration (variation: 0.02) per sq.m. The various categories of specimens, collected at the Trou d'eau site were: N = 87.5%, P = 10.4% and Pa = 2.1%.

The fission rate (F%) at the Trou d'eau site as extrapolated from the figures for the different categories of specimens was 5.2 per cent. The regeneration rate (R%) was 4.3 per cent.

The difference in numbers between P and A specimens suggest that mortality among A specimens is clearly higher than that of P specimen (not a single A specimen was found at this site). In their natural environment, specimens in the process of fission (F) were mostly found under coral heads (*Poritidae*), where they hide in order to protect themselves from possible predators.

### Anatomy in the different categories of specimens

#### 'Normal' specimens (N) (Figure 1)

*H. leucospilota* is a large sea cucumber with an average length of 18 cm and an average total open weight of 245 g at the study site.

Its integument is very thick, i.e. 8 mm. The weight of the integument (muscles included) averages 82 g. Normal specimens are characterised by five pairs of longitudinal muscles which are attached near the peripharyngeal calcareous ring at the mouth and at the cloaca in the posterior area. Each pair of muscles has an average width of 8 mm.

The mouth opens in the anterior part and has twenty oral tentacles. The intestine is filled with sand, dead coral, and other rock debris and has a thin integument. The intestine is divided into three loops. The average length of the intestine of the five specimens studied was 98.4 cm. The transverse vessel joins the first and the second loops. The intestine is attached to the integument by a number of mesenteries. The *rete mirabile* is found within the second loop of the intestine and takes part in intestinal absorption.

Twenty vesicles of oral podia were located around the calcareous ring and provide turgescence to the oral tentacles. The average length of the oral podia in normal specimens was 2.5 cm. The Polian vesicle below the calcareous ring is large in *H. leucospilota* (average length 4.9 cm). *H. leucospilota* is gonochorismal. The gonads were attached along the mesentery by the gonad base, approximately 4.5 cm from the mouth, and had the form of a group of branched tubules.

At maturity, male and female gonads can be differentiated in *H. leucospilota* by their colour, as male gonads are beige, while female gonads are bright pink in colour. For the five individuals specimens studied, weight of the gonads varied between 9 and 32 g, with an average weight of 18.9 g.

The gonadal-somatic ratio was between 12.5 and 33.86. The respiratory organs consisted of two respiratory trees, i.e. the right and left. Each respiratory tree consisted of a trunk with branches, attached very high on the anterior part and opening onto the cloaca. Diffusion of water by means of these organs is a passive phenomena, permitting respiratory exchanges and the elimination of metabolic wastes.

The right respiratory tree was attached to the integument by the mesentery while the left respiratory tree was attached to *rete mirabile*. When this species is attacked, it releases a great number of very fine Cuvierian tubules from its anus. The tubules are normally carried on the base of the respiratory trees (Van den Spiegel, 1994).

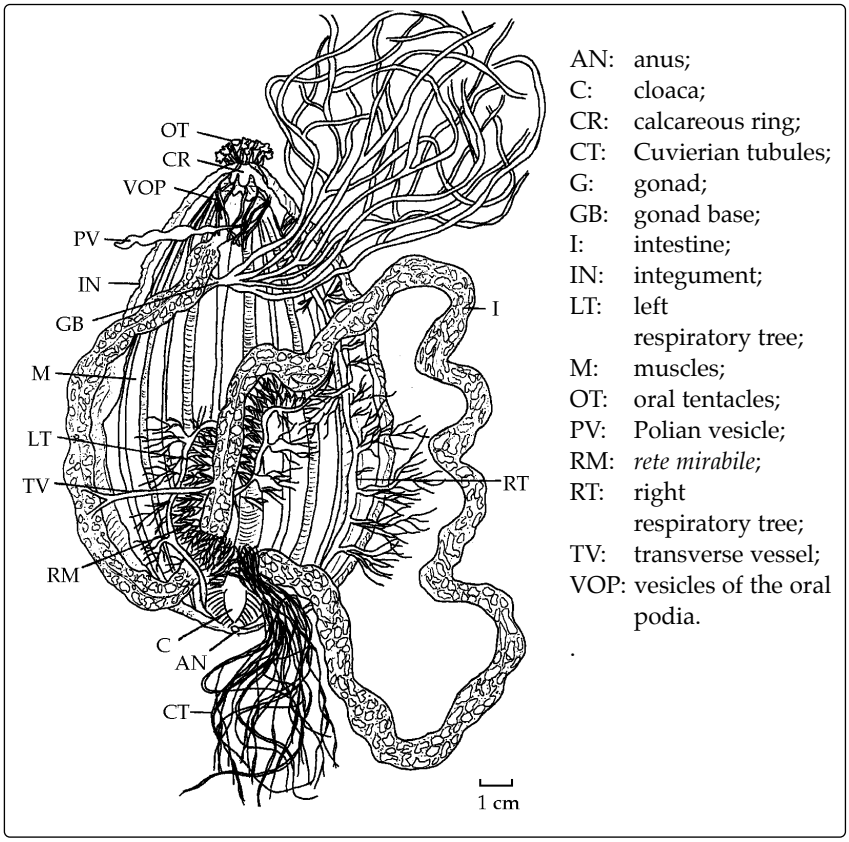


**Figure 1: Dissection of an N specimen**

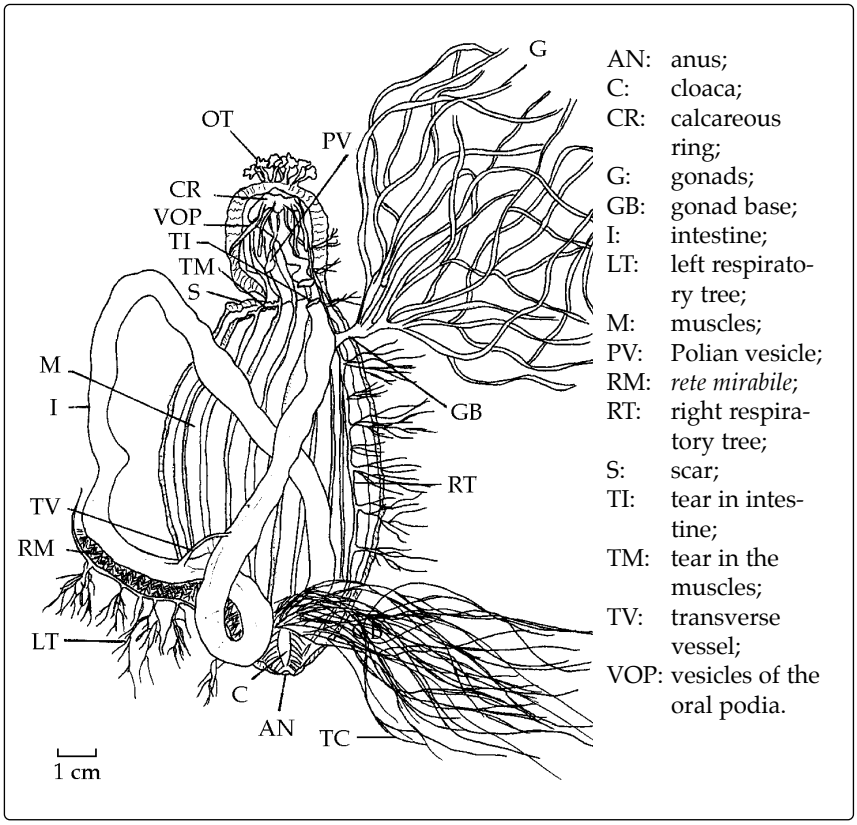


**Figure 2: Dissection of a specimen in the process of fission (F)**

### *Specimens in the process of fission and regeneration*

*Specimens in the process of fission (F) (Figure 2)*

All these specimens showed constriction in the anterior part of the body. During the early stages of fission, constriction is annular. Ambulacral papillae disappear from the integument at the constriction ring. During advanced stages, constriction leads to a rupture in the integument and organs, thereby separating the sea-cucumber's body into two parts. These two parts are still attached by a thin ventral integument. In certain F specimens, regeneration can begin as early as this stage. In fact, some specimens showed regeneration of the mouth and oral tentacles while the two parts of the specimen in process of fission were still attached. Figure 2 shows the anatomy of an F specimen.

In *H. leucospilota*, fission took place in the anterior part of the body at about 19 per cent (1/5) of the total length of the specimen. The average thickness of the integument in the anterior half was 8 mm and thinned out near the mouth. The longitudinal muscles were thin and measured on average 2 mm in width in the anterior part. The digestive system was always empty in the anterior part, which may be explained by the fact that the animal no longer feeds during fission. The Polian vesicle averaged 15.8 mm, and this size is much smaller than in normal specimens. The oral podia had the same size as normal specimens.

Only the right respiratory tree was found in the anterior section. It was always located near the calcareous ring and was connected to the integument. The integument wall was thin in the area where fission had occurred, i.e. an average thickness of 2 mm. The muscles were torn apart at the moment of fission. During the initial stages of the fission, the intestine was constricted and during the final stages, it was sundered. In the anterior half, it was attached to the pharyngeal

bulb. Only the right respiratory tree is affected by fission. It breaks in such way that part of it remains in the anterior part, so most of the respiratory tree was located in the posterior section.

The integument on the posterior half was thick, and muscles were much more voluminous than in the anterior part. The major portion of the intestine was found in the posterior region and was filled with fine coarse sand. This sand accumulated during the later stages of fission. Gonads were not always present in specimens undergoing fission. Of the five F specimens studied, only two specimens had gonads. The average weight of the gonads in F specimens was less than the weight of gonads in normal specimens, i.e. 1.3 g.

*Anterior specimens (A) (Figure 3)*

A specimens corresponded to the anterior part which broke off during fission. The integument formed a more or less closed scar depending on the amount of time which had passed since fission.

On average, A specimens measured 43 mm and had a weight of 9.6 g. The integument had an average thickness of 3 mm, and thinner near the scar (1.5 mm). During the initial stages of regeneration, the scar was open, while in later stages, it was completely closed. There were muscular bands in the area near the scar, while the muscles were attached normally out the calcareous ring. The intestine had an average length of 22.5 mm and weight of 0.11 g and was empty.

In A specimens, the intestine did not show any anal orifice. Only the right respiratory tree was present and was attached to the integument without any sign of regeneration. The Polian vesicle was small (average length: 2 mm) in comparison to that of normal specimens. The oral podia were smaller in size than those of N specimens. As A specimens do not feed, the oral tentacles were not functional and appeared atrophied. The gonad base was visible in P specimens, but gonads were absent in A specimens.

*Anterior specimens in the process of regeneration (Ap) (Figure 4)*

Ap specimens were characterised by regeneration of the posterior part. The size of the regenerated part depended on the amount of time which had passed since fission. In the Ap specimens studied, the length of the regenerated part varied from 5 to 20 mm. The regenerated integument was thin and could have ambulacral papillae. The average thickness of the regenerated integument was 1 mm. Regeneration of the muscles in the posterior part of Ap specimens began at the muscular band. The regenerated muscles were thinner and were attached to the integument at the cloaca. All Ap

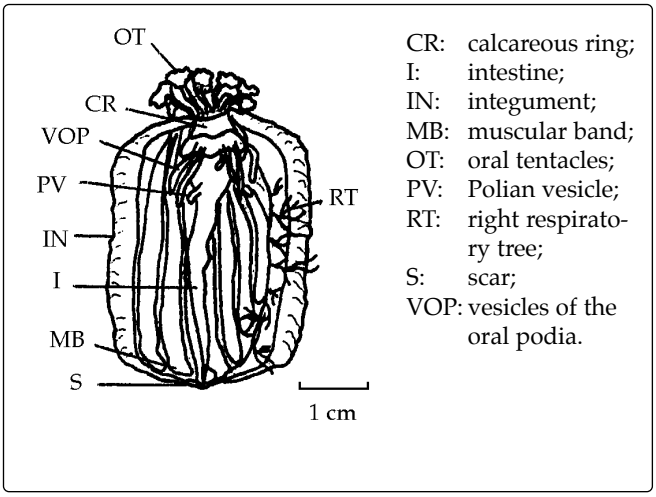


**Figure 3: Dissection of an A specimen**

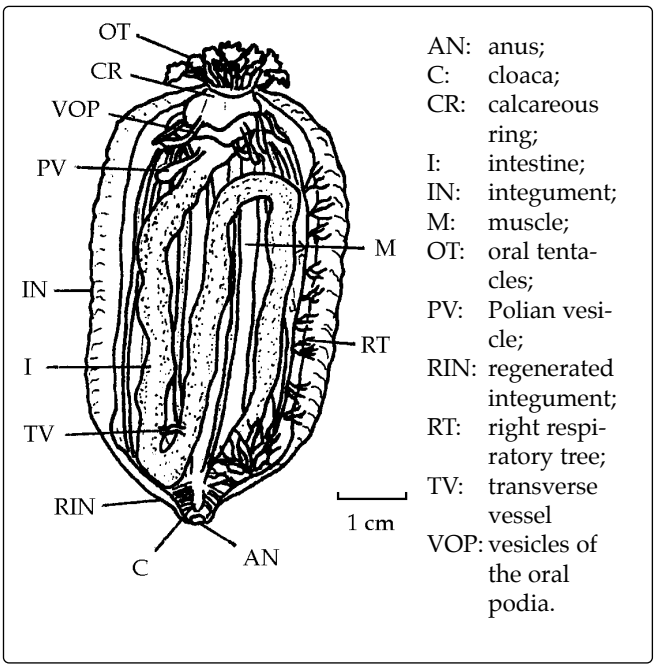


**Figure 4: dissection of an Ap specimen**

specimens showed some regeneration of the intestine and anus. Regeneration of intestine involved regeneration of the transverse vessel. The intestine appeared to begin to function as soon as the anus had regenerated since after that time it was filled with sand. Complete regeneration resulted in an intestine with three loops and a transverse vessel and which had a cloaca. When fission had taken place quite recently, only the right respiratory tree was found. It was also one of the first organs regenerated and extended as far as at the cloaca. In those instances where a significant amount of regeneration had taken place, the left respiratory tree regenerated on the intestine. The turgescence of oral podia indicated that they were functional. The Cuvierian tubules regenerated as soon as the intestine was fully formed, i.e. even before the cloaca. Gonads did not regenerate in Ap specimens.

*Posterior specimens (P) (Figure 5)*

P specimens correspond to the posterior part of the sea cucumber after fission. Cicatrisation and internal anatomy depended on the amount of time which had taken place since fission. When fission was recent, the scar was open, leaving the intestine visible. In later stages, it was completely closed but there was no oral orifice.

The length of the P specimens observed ranged from 110 to 180 mm, while the total open body weight varied between 89 and 104.5 g. The integument was very thick with an average thickness of 7 mm. Thickness dropped drastically at the scar site; i.e. to 2 mm. In some instances, folding of integument closed off the scar. In P specimens with recently-formed scars, the muscles were torn and formed bands at the end. In all the P specimens studied, the intestine consisted of three loops with a transverse vessel and was open at the scar. When the scar is open, the intestine was in contact with the outside environment. As P specimens do not feed, the intestine was always empty.

In some P specimens, the intestine showed signs of regeneration with a very thin integument, while in other cases, it desintegrated and did not show any signs of regeneration. If the intestine had not become atrophied, the entire *rete mirabile* was present. Both respiratory trees were found in P specimens. The left respiratory tree was not affected by fission and was still linked to the *rete mirabile* whenever the latter was present. The right respiratory tree was broken during fission but could be found in the posterior part. The Polian vesicle was absent in all P specimens. Some specimens had a ring of oral podia at the scar site even when the mouth had not yet formed. Of the five P specimens studied, only one had gonads which were small in size. They were found on a specimen which had just undergone fission. All P specimens had Cuvierian tubules.

*Posterior specimen in the process of regeneration (Pa) (Figure 6)*

Pa specimens corresponded to P specimens which had regenerated their anterior part, including their mouth and oral tentacles.

The integument had an average thickness of 6 mm, while in the regenerated section, the integument only measured 2 mm. Significant regeneration of the anterior section involved regeneration of the first ambulacral papillae.

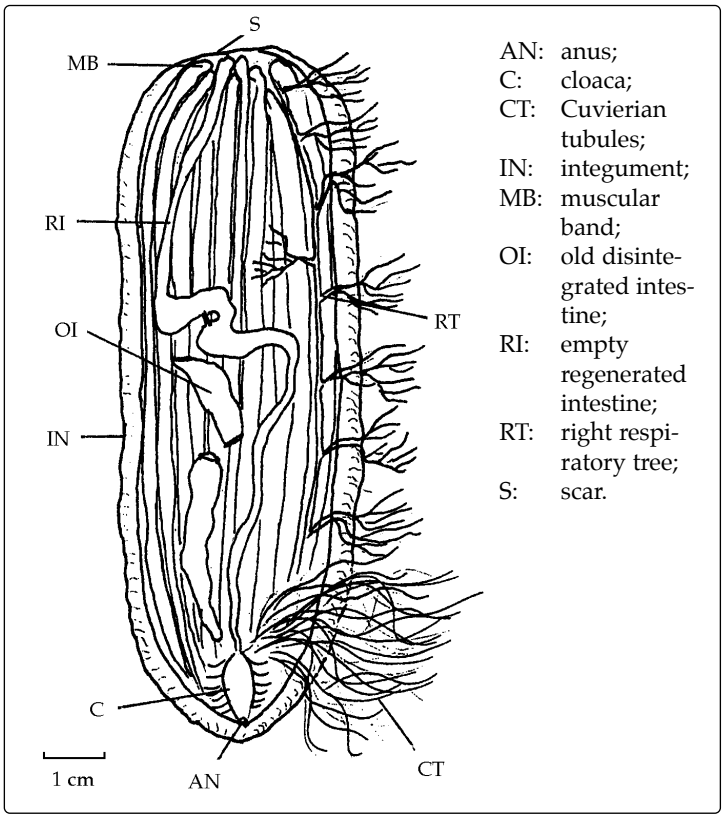


**Figure 5: Dissection of a P specimen**

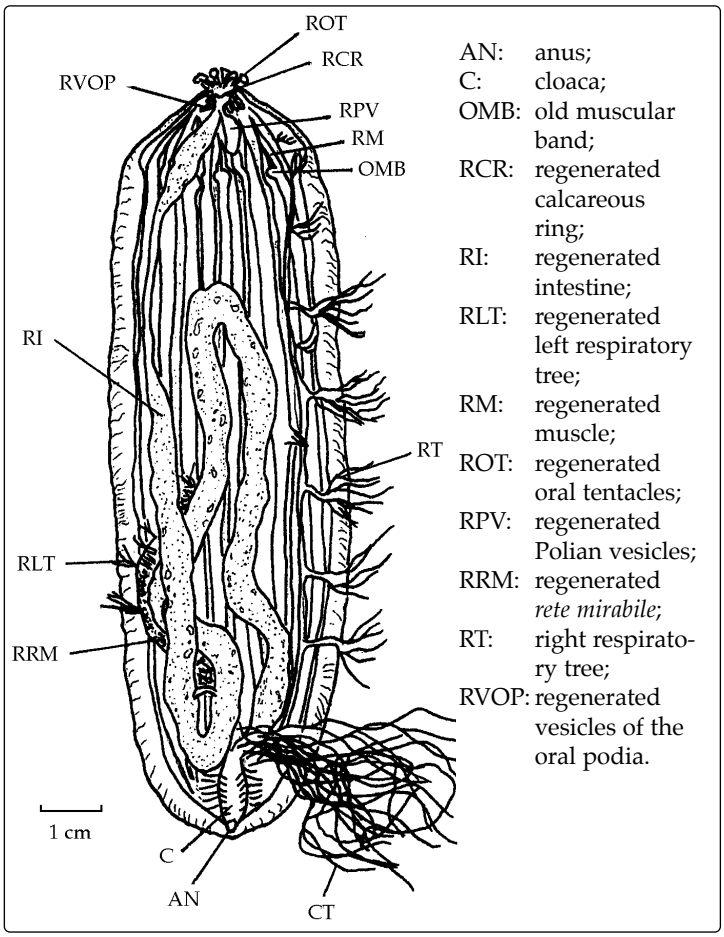


**Figure 6: Dissection of a Pa specimen**

One pair of muscles had an average width of 6 mm, while in the regenerated part this width was only 2 mm. In P specimens, regeneration of the muscles in the anterior part began with the muscular bands near the scar. The intestine began regeneration at the mouth which was surrounded by a ring of completely-regenerated oral tentacles.

In all Pa specimens, the intestine was filled with sand. Feeding began as soon as the mouth had been regenerated. Mainly the anterior part of the intestine was regenerated. The oral podia and Polian vesicle were regenerated in Pa specimens and turgescence of the vesicles indicated that they were functional.

Both respiratory trees were present. Only the right respiratory tree regenerated into the anterior area. None of the Pa specimens had gonads. As in P specimens, the Cuvierian tubules were always abundant in Pa specimens, and did not need to be regenerated.

## Discussion

### *Population characteristics*

At the Trou d'eau site, *H. leucospilota* density in the back reef was 0.96 specimen per $m^2$. In Trois-Chameaux and Planch'Alizés sites, *H. leucospilota* density was respectively 0.5 specimens and 1.2 specimens per $m^2$.

Density levels for the species *H. atra* are generally higher and can reach 4 specimens per $m^2$ (Conand, 1996). As with *H. atra*, the highest density levels for *H. leucospilota* were observed in the back reef. However, *H. leucospilota* was also found on the reef flat where hydrodynamics are strongest.

In the Trou d'eau site, although actual numbers were low, taking into account all A, P, Ap and Pa specimens, 12.5 per cent of the total specimens were a result of asexual reproduction.

The fission rate (F%=(A+P)/2T*100) was 5.2 per cent for *H. leucospilota* at the Trou d'eau site, which is low compared to *H. atra* whose fission rate in the back reef is 20 per cent (Conand, 1996). Data on fission rates for *H. atra* (Conand, 1996) depend on the area of the reef which is studied.

The regeneration rate (R%=(Ap+Pa)T*100) was 4.3 per cent for *H. leucospilota,* while it is 10.6 per cent for *H. atra* . In *H. leucospilota,* the regeneration rate was lower than the fission rate, which indicates some mortality of specimens after fission.

The percentage of Pa specimens (20%) was clearly higher than that of Ap specimens (0%), and this corroborates Doty's hypothesis for *H. atra* (1977), i.e. that the mortality of Ap specimens is higher than the mortality of the Pa specimens.

### *Fission and regeneration results / comparison to* H. atra

In *H. leucospilota*, fission occurs much farther back on the body than it does in *H. atra*. Fission in *H. leucospilota* occurs at 19 per cent of the total length while for *H. atra* it occurs at 45 per cent of the total length (Conand & De Ridder, 1990). Fission may be controlled by different factors. Hydrodynamics, immersion, temperatures, salinity, solar radiation and desiccation may be responsible for fission in *H. atra* (Doty, 1977; Conand & De Ridder, 1990).

In the La Saline Reef, man-made changes like eutrophisation of the water or sea cucumbers being stepped on may be possible catalysts for fission (Conand, 1996). *H. leucospilota*'s intestine is empty above the fission point but is filled with sand in the posterior part. The right respiratory tree is affected by the constriction with the largest part of this tree found in the future P specimens, and only a fragment remaining in the anterior part.

The same phenomena was observed in *H. atra* (Conand, 1996). Specimens in the process of fission (F) do not always have gonads. When they do, the gonad base is situated after fission in the posterior part in *H. leucospilota* while it is found in the anterior part in *H. atra*. Only P specimens of *H. leucospilota* have gonads, whereas in *H. atra*, it is the A specimens which have them.

After fission, A and P specimens are characterised by a scar located in the posterior part of the A specimens and in the anterior of the P specimens. The intestines of A and P specimens are always empty as they do not feed. Muscles formed bands near the scars and regeneration begins from these bands.

As for regeneration, the length of the part regenerated depends on the amount of time which has occurred since fission. The thickness of the regenerated integument is always less than that of normal integument. As with *H. atra*, *H. leucospilota* can show ambulacral papillae on the integument if the regenerated part is significant.

Regenerated muscles are thin and fine and converge either towards the anus (in Ap specimens) or the mouth (in Pa specimens). The intestine of *H. leucospilota* seems to regenerate and begin normal functioning rather quickly as is the case with *H. atra*. In any case, complete regeneration leads to the appearance of a mouth, three intestinal loops, a transverse vessel and anus.

The first stage in the regeneration of digestive organs in Ap specimens is regeneration of the anus. Next the intestine lengthens to form three intestinal loops. Regeneration of the transverse vessel connecting the first loop to the third one occurs rather early. The *rete*

*mirabile* regenerates on the second intestinal loop. As soon as the anus is open, the intestine becomes functional. The defence organs develop after regeneration of the cloaca. The right respiratory tree regenerates from a fragment of the respiratory organ found in A specimens. The left respiratory tree develops later.

In Pa specimens, regeneration first involves the anterior part of the intestine. The oral podia develop very early before perforation of the mouth. When the mouth has formed, it is surrounded by a ring of oral tentacles. The Polian vesicle forms. The old intestine from P specimens disintegrates and disappears.

Pa specimens then regenerate an intestine with three folds, a transverse vessel and a *rete mirabile*. Pa specimens which have oral tentacles and a functional digestive system can feed. The right respiratory tree is maintained and only regenerates the anterior part in Pa specimen. Defence organs show no signs of regeneration.

*H. leucospilota* is gonochorismal. All the specimens collected in February had gonads. As the gonadal-somatic ratio was high (22.6), sexual reproduction can take place at this time of the year. Specimens in the process of fission collected at that time of the year also had mature gonads, so during periods of sexual reproduction asexual reproduction can also take place.

## Conclusion

This study has proven the existence of fission in *H. leucospilota*. The chronology of the regeneration of organs has been established. Fission for *H. atra* has been the object of more serious and detailed studies on the fission and regeneration rates than has been the case for *H. leucospilota*.

It would be interesting to analyse the role of asexual reproduction in population dynamics for this species and to know if environmental factors play a role in the fission rate.

Is asexual reproduction in this species determined by specific factors, in particular, environmental disturbances which occur frequently in the reefs of Reunion Island?

# Fishery in Washington State

*by Alex Bradbury*

The commercial dive fishery for *Parastichopus californicus* in Washington State, USA, underwent management changes in 1993 following court decisions granting Indian treaty tribes the right to take half of the harvestable resource.

The former management policy of rotating fishing areas such that each area was fished only once every four years had to be abolished in 1994 to allow treaty tribes to harvest annually within their traditional fishing areas. Quotas were reduced roughly one-quarter in each area to accommodate the yearly fishery.

Following this change in management practices, logbook data from non-Indian divers has been monitored to detect shifts in either catch-per-unit-effort (CPUE) or mean harvest depth. Three seasons have elapsed since the management shift to yearly quotas: 1994, 1995 and 1996.

Mean CPUE and its variance is derived from log normalised diver logbook data and is reported as kg of split, eviscerated, and drained sea cucumber per hour of diving. For the 1994, 1995 and 1996 fishing seasons, CPUE has been 66 kg, 64 kg, and 61 kg per diver-hour, respectively. There was no statistically-significant difference between these CPUEs for the last three seasons (coefficient of variation or CV for the three means was 2.2%, 1.9% and 1.9% respectively).

Mean harvest depth for the 1994, 1995 and 1996 fishing seasons was 15.7 m, 16.4 m and 14.6 m, respectively.

This preliminary analysis suggests that there have been no significant changes in either mean catch rate or mean harvest depth thus far following the shift from rotational area management to yearly statewide fishing in all areas. However, Washington Department of Fish and Wildlife will continue to monitor these and other fishery-dependent indicators to determine if current harvest levels are sustainable.

Underwater video surveys of sea cucumber density will be performed again in the summer of 1997 throughout the most important fishing area, the San Juan Islands.