

While reseedling may be a viable option to assist rehabilitation in future management agencies are more concerned with the effective sustainable management of other commercial species being fished at present.

Recent catch estimates

Combined log returns from individual islands indicate that 15 tonnes of prickly redfish, 23 tonnes of black and white teatfishes, and 12 tonnes of all other species were harvested during 1999. The 1998 catch included 80 tonnes of prickly redfish, 20 tonnes of teatfishes and 15 tonnes of all other species combined. The 1997 catch comprised 57 tonnes of prickly redfish, 29 tonnes of teatfishes and 29 tonnes made up of all other species combined. These figures are in wet weight and gutted.

Official figures for 1995 revealed the total harvest of sandfish was around 1000 tonnes. Industry estimates place the total catch between 1200 and 1400 tonnes wet weight, with all but approximately 50 tonnes being sandfish.

Sexual reproduction of *Stichopus chloronotus*, a fissiparous sea cucumber, on Reunion Island, Indian Ocean

Status of other commercial species

The status of black and white teatfish, surf redfish and other lower value species remains unknown at present. It is possible these species may be the target of increased fishing pressure in future due to the growing export market demands for quality beche-de-mer.

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Introduction

Stichopus chloronotus is a holothurian of the order Aspidochirotetes, family *Stichopodidae*, that is widely distributed across the tropical Indo-Pacific region. It is mainly found on reef flats and slopes with considerable hydrodynamic energy. The species' density is relatively low, but sometimes reaches up to several specimens per m² (Franklin 1980; Conand 1989; Uthicke 1994; Conand et al. 1998).

Similarly to nine other Aspidochirotetes sea cucumbers, they can reproduce both sexually and asexually (Uthicke 1994, 1997, 2001; Conand et al. 1998). Their sexual reproduction has been studied in Australia (Franklin 1980) and Malaysia (Tan Shau-Hwai and Bin Yasin 2000). Asexual reproduction is achieved by transverse fission resulting in two animals that each regenerate the missing part (Uthicke 1997; Conand and Uthicke 1998; Conand et al. 1998).

The aim of this study was to describe the sexual reproduction cycle on Reunion Island. The results

should provide a better understanding of the respective roles of sexual and asexual reproduction and facilitate the interpretation of population genetics (Uthicke et al. 1999 and 2001).

Materials and methods

Sites

The Trou d'Eau station is located on Reunion Island's west coast on the Saline-les-Bains reef complex that spans five kilometres. It is a fringing reef that is swept by the trade winds, but with little hydrodynamic activity. Most sampling was conducted on this site, which is a back reef forming a channel made up mainly of detrital coral sediment littered with large basalt blocks. Small amounts of brackish water well up into this 0.70-m deep area at the shoreline, providing algal cover for the substrate at certain times of the year.

Seawater surface temperature was selected as a reference parameter and recorded hourly throughout the study, ie from March to April 2001, using a

VEMCO minilog-T sensor set three metres deep at the Pointe des Galets harbour exit (F. Conand pers. corr.). The temperature curve throughout the study was obtained by five-day averages of hourly readings.

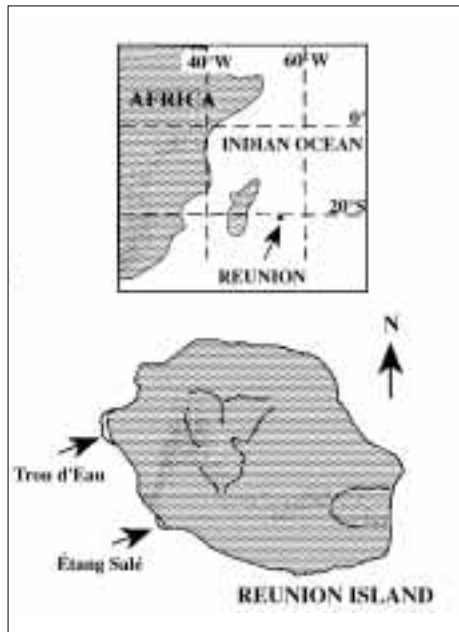


Figure 1. Sampling sites of *Stichopus chloronotus*

Sampling

Twenty adult specimens were collected monthly at the Trou d'Eau station from March 2000 to April 2001. They were selected from among the longest and heaviest category, so as to avoid any bias arising from the relation between size and the gonad index value (Franklin 1980). Such sampling based on a uniform size category is recommended for studying reproduction cycles. Small specimens were also sampled to determine the population's size at first sexual maturity. Further sampling was conducted at the Planch'Alizés station (Fig. 1), following early results, so as to obtain extra females.

Gonad dissection and processing

Specimens were anaesthetised in a 37 % magnesium chloride solution and dissected on arrival at the laboratory after a one-hour journey. The length to within 0.5 cm (Lt) and total weight to within 10^{-1} g (Wt) were measured for each specimen. The gonads were weighed to within 10^{-2} g (Wg) and fixed in formalin (10 %). Finally, the eviscerated weight (We) was obtained, which is more representative than the total weight that includes the coelomic fluid and digestive tract contents, which cause considerable variability (Conand 1989). The gonad index or

GI follows the Conand (1989) calculation method. The monthly average (+/- standard deviation) was calculated and the patterns indicated maturity, spawning and sexual resting periods.

$$GI = (100 \times Wg) / We$$

Stichopodidae gonads have two tufts of tubules located on either side of the mesentery. The tubules branch out distally and are joined at the base in a sac, which bulges out from the dorsal mesentery. Saccules, ie dilations developing during maturation, are generally observed in this family (Conand 1993a). This characteristic form is not found, however, in *S. chloronotus*, which has tubular distal tips.

The gonads' macroscopic and microscopic characters were determined using samples fixed in formalin. The lengths of gonad tubules were measured from the gonad base to the distal tip to within 5 mm (Lg). Tubule diameters were also measured (Dg). Both values were used to describe *S. chloronotus* maturity stages by comparing them with macroscopic gonad characteristics such as colour, morphology and consistency and by using other descriptive methods, such as gonad indices, microscopic observations and histological sections.

It was possible to determine the sex in females during advanced stages of maturation using a binocular magnifier, but it was generally necessary to resort to a microscope. Also, oocyte diameter distribution was established based on samples preserved in formalin and was used to determine maturity stage characteristics.

Atresia was related to undischarged germ cell disintegration. These germ cells were, therefore, observed as coloured clusters in several areas of the gonad tubule lumina. A new parameter was defined and used to determine the end of the sexual cycle, because the proportion of clusters increased as reproductive activity declined. A semi-quantitative value or atresia index was allocated to each specimen using the formalin samples.

- *no atresia* (0): no degenerative cell clusters observed anywhere in the gonad
- *low atresia* (1): small scattered coloured clusters mainly distributed at the tubules' distal tips
- *medium atresia* (2): much larger and more numerous clusters, still mainly concentrated in the distal tips. Clusters clearly visible to the naked eye.
- *high atresia* (3): clusters replaced by structures taking up the entire lumen volume in the gonad's main tubules. Some clusters remained in the distal tips of tubules that appeared fairly empty.

An atresia development curve was obtained and correlated with mean GI curves throughout the study.

Histological sections were prepared to provide more accurate descriptions of the observed maturity stages. Two stains were used, ie Trichrome and HPS (hematoxylin-phloxin-saffron).

Results

Sex ratio

Out of the entire year's sampling of 260 specimens, only 8 females were collected, giving a sex ratio of 97.3% males (Fig. 2). In the extra sample collected at Planch'Alizés, 5 of the 12 specimens obtained were female and 7 male, which was a more balanced sex ratio.

Gonad and gonad index study

Monthly mean GI variations (Fig. 3a) indicated several phases in the year. From March to late May, values remained fairly stable between 1.64 and 1.69. From late May onwards, a sharp GI fall was observed, reaching 0.83 ± 0.29 in July. A gradual return to the mean GI was then noted, followed by a maximum in early November at 3.22 ± 0.93 , ie the first GI peak. Gonad indices subsequently fell slightly up until late December (2.41 ± 1.36). In January, GI rose very steeply again to reach a second peak in late January (4.58 ± 1.36). Values then gradually declined until late March (3.31 ± 1.10). Two peaks were therefore noted on the GI curve, ie around mid-November and late in January. Falling values coincided with the two spawning seasons (Conand 1989). Both peaks occurred in the warm season (Fig. 3b), when seawater temperatures were

at their highest. GI values obtained for March (Fig. 3a) differed from one year to the next, however, with a mean GI in 2000 of 1.64 ± 0.45 and for March 2001 of 3.31 ± 1.10 ($t = -6.27$, 5% significant difference threshold).

An analysis of gonad tubule diameters indicated that this parameter generally followed the mean GI curve. From March to June, tubule diameters slowly narrowed at the same time as gonad indices dropped off for the first time. When gonad indices rose to their initial peak in mid-November, tubule diameters increased considerably. As with diameters, gonad tubule lengths also varied in line with the monthly GI curve. Average lengths dropped with the first GI trough and rose again with the first GI peak.

Providing a macroscopic description of gonads in terms of the maturity stage was more difficult than with other sea cucumber species (Conand 1989, 1993b). There was no major variation in appearance during the sexual cycle. The following phases were nevertheless identified: 1) a maturing stage; 2) a pre-spawning or ripe stage; and 3) a post-spawning stage (Fig. 4). No immature specimens were observed, other than in the extra sampling conducted to determine weight at first maturity.

With regard to atresia, the mean atresia index curve during reproduction (Fig. 3a) generally followed the opposite path to mean gonad indices. When the initial GI peak occurred in mid-November, atresia was falling sharply, reaching nil in late November. After rising steeply again in January, between the two spawning seasons, it declined once again. Mean atresia index increases occurred during GI falls, immediately after spawning.

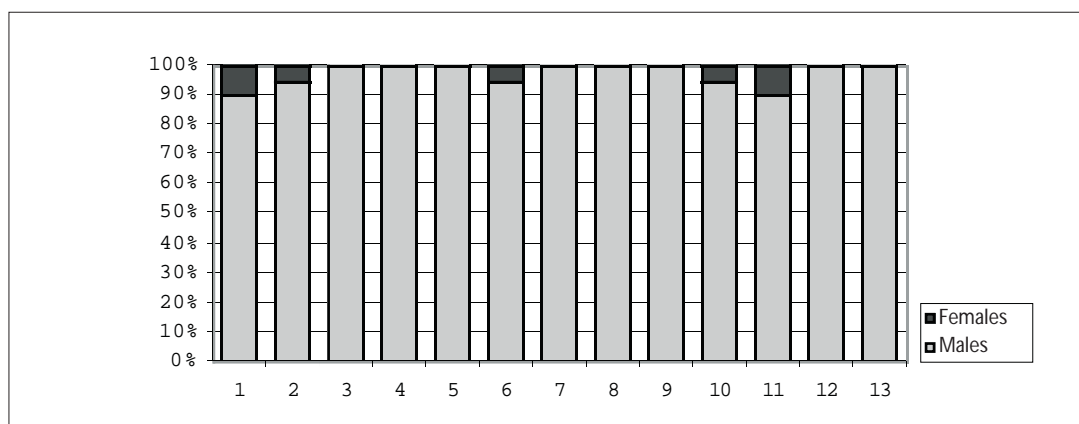


Figure 2. Monthly sex ratio in *Stichopus chloronotus* population from Trou d'Eau, La Reunion (monthly sample of 20 individuals, March 2000 – April 2001)

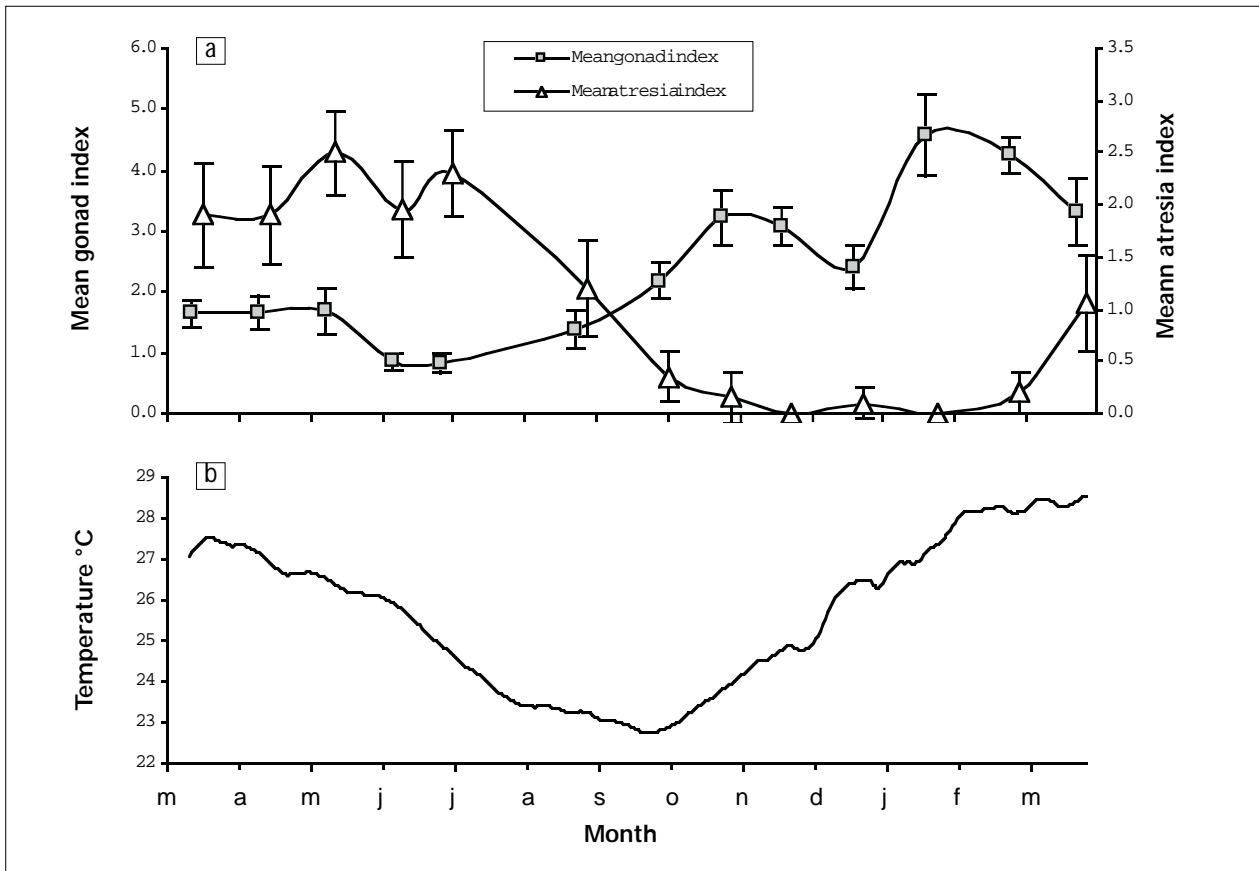


Figure 3. Monthly variations of gonad index and atresia index (\pm SD) of *Stichopus chloronotus* and sea water temperatures (La Reunion)

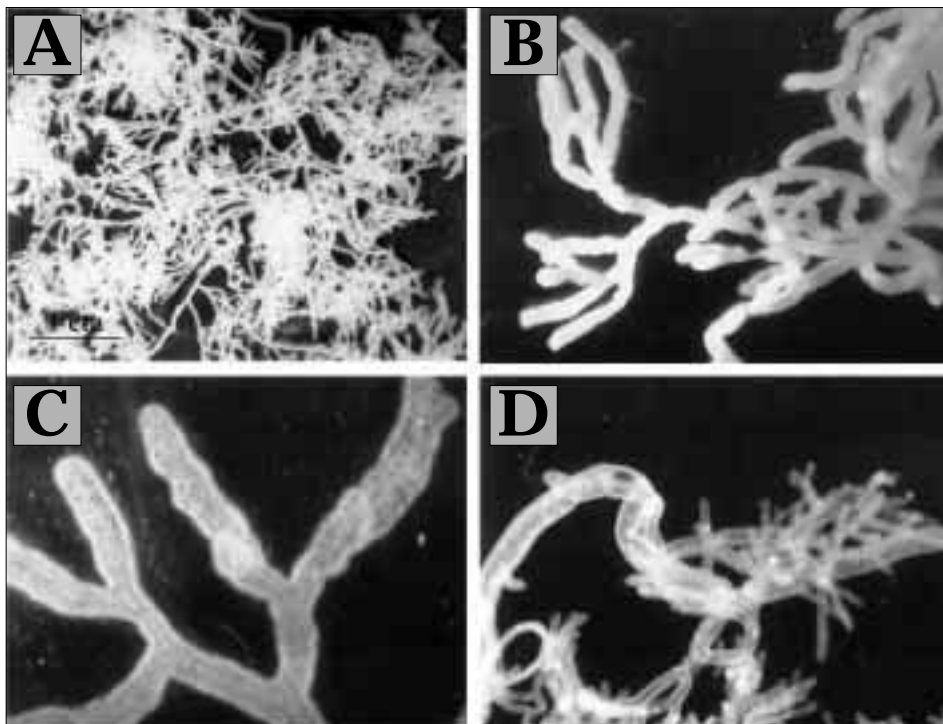


Figure 4. Morphology of *Stichopus chloronotus* gonads.
 A: Testis. B: Mature testis tubule.
 C: Mature ovarian tubule. D: Post spawning testis showing atresia.

The oocyte diameter frequency distribution based on formalin samples is presented for each stage in Figure 5. During the post-spawning stage (Fig. 5a), oocytes were distributed between 12 μm and 48 μm , the mode being 30 μm . During maturation (Fig. 5b), developing oocytes have diameters ranging from 36 μm to 78 μm , the average being 64 μm . The only three females collected during the spawning season (Fig. 5c) had oocytes measuring 30 to 114 μm with the mature oocyte mode being 84 μm .

A more accurate appreciation of these stages was obtained through the histological study. It provided a clearer distinction between immature and maturing specimens. Four maturity stages were described using the histological approach, i.e. 1) immature; 2) maturing; 3) pre-spawning or mature; and 4) post-spawning (Fig. 6). These stages are described in Table 1.

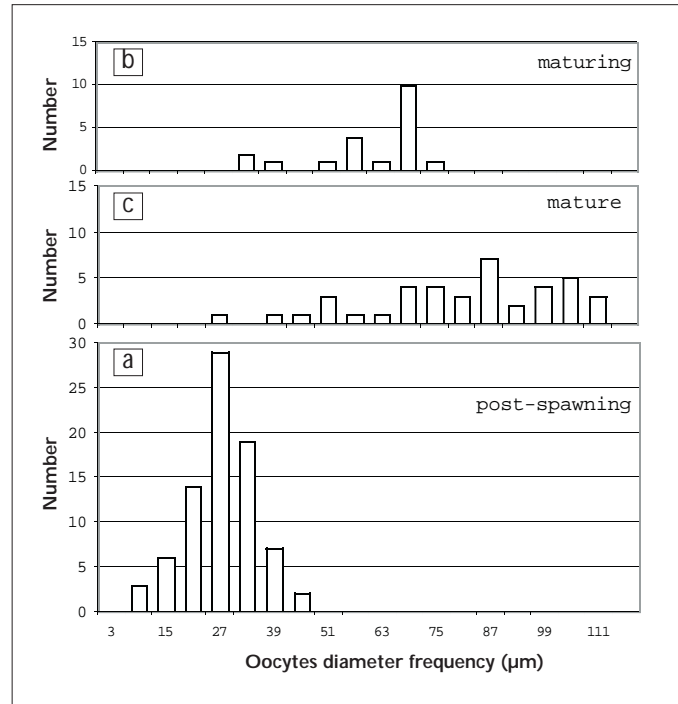


Figure 5. Oocyte diameter frequency distributions of *Stichopus chloronotus*

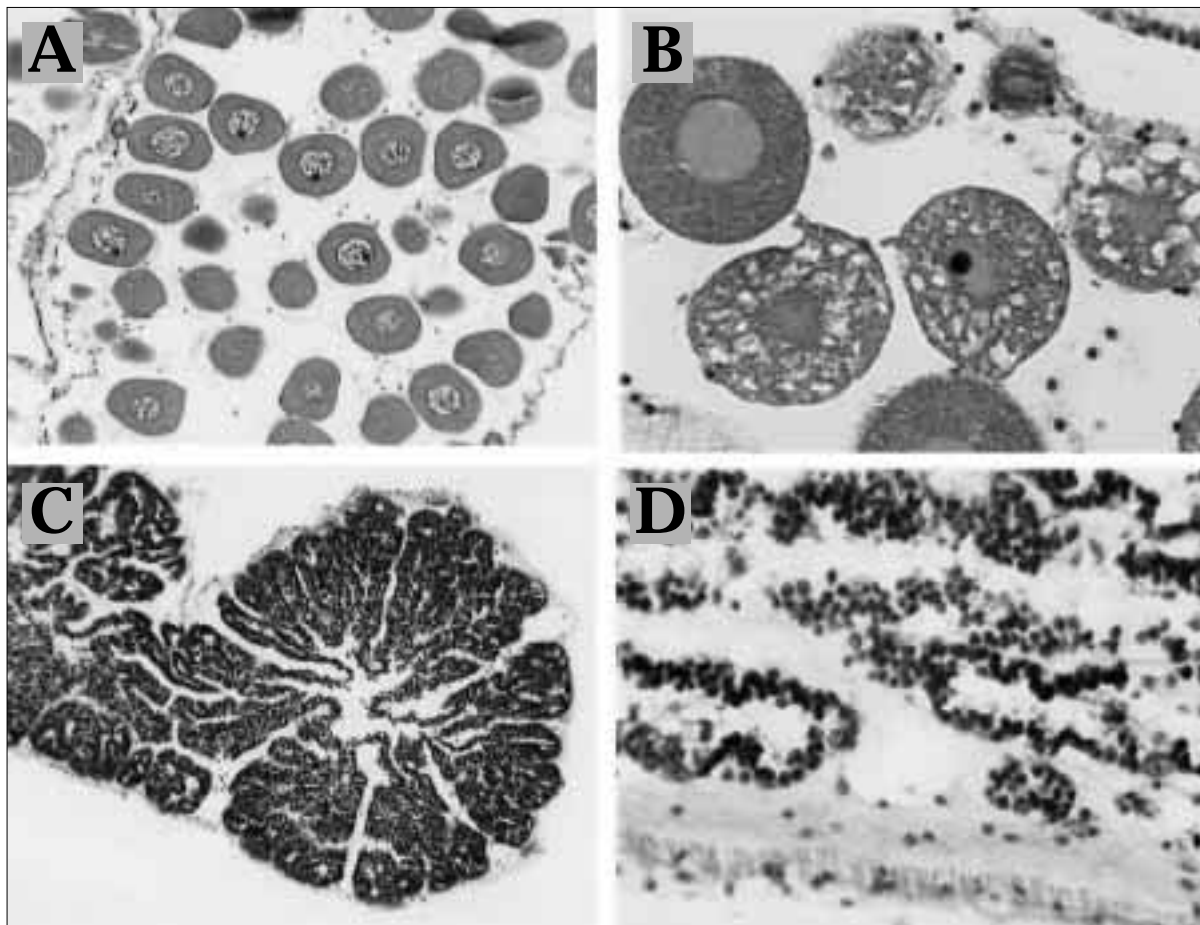


Figure 6. Histological characteristics of *Stichopus chloronotus* gonads.
A. Ovary, vitellogenic oocytes. B. Post spawning ovary with degenerating oocytes.
C. Mature testis with spermatocytes and sperm. D. Post spawning testis.

First maturity

First maturity was determined on the basis of 20 small specimens from the extra samples collected at Trou d'Eau (11/03/01). Weight at first maturity was estimated at 50 g. All specimens weighing more than 50 g proved to have gonads, while those weighing less did not.

Maturity stage variations during the yearly cycle

Figure 7 presents the proportions of specimens at each stage over time supported by the various methods. Most post-spawning specimens were observed from March to July and declined as maturing specimens increased from August to October.

Pre-spawning specimens rose sharply in November and fell in December. They increased abruptly again in January and gradually waned in February and March. The cycle was therefore characterised by a minor initial spawning season in November, followed by the main season in February/March during the warm season.

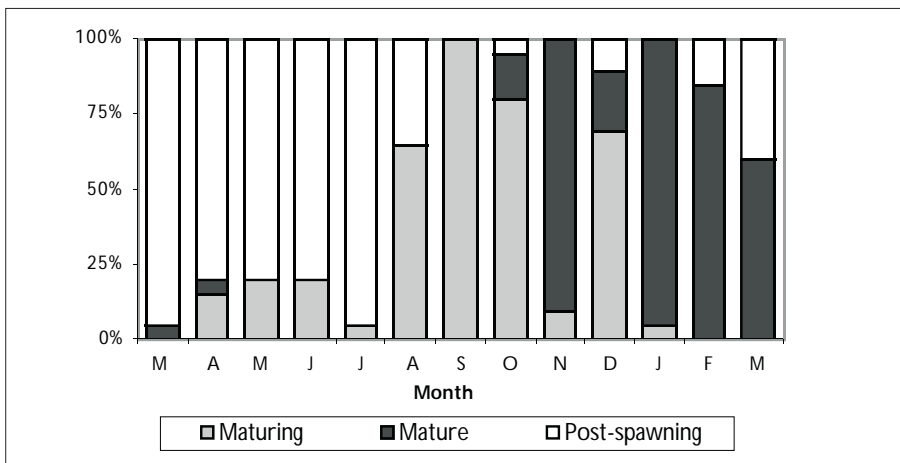


Figure 7. *Stichopus chloronotus*'s reproductive cycle on Reunion Island

Table 1. Macroscopic and microscopic characteristics of *Stichopus chloronotus* gonads at different maturity stages

Various stages	Gonad anatomical and macroscopic characteristics	Microscopic characteristics
Immature stage	Small tubules with little branching	Some sparse villi against tubule walls. Few germ cells.
Maturing stage	Whitish tubules already branching out considerably; villi clearly visible	Males: Increasing villi. Few germ cells in tubule lumina. Females: Oocytes tightly packed and filling entire tubule lumina. At this stage, oocytes measure 36 to 78 µm, the average being 64 µm.
Mature stage	Slight colour change from whitish to off-white and even creamy brown in both sexes. Tubules are bulging.	Males: Villi are less prominent and remain on walls. Maturing spermatocytes are clearly visible. Sperm appear in the form of thick granules in the tube lumina. Females: Oocytes are not attached to tubule walls, which still hold oogonia. At this stage, most oocytes are mature, measuring an average 84 µm with the largest reaching 114 µm. They have a very wide, clearly visible nucleus and an eccentric nucleolus.
Post-spawning stage	Tubules are more or less empty, but have residual, undischarged sperm or oocytes undergoing atresia (yellowish clusters)	More or less empty tubules are observed in both sexes. Undischarged cells and oocytes at various stages of deterioration are noted in females and atresia clusters in males.

Discussion

This study follows on from an analysis of asexual reproduction by fission in *Stichopus chloronotus* (Conand et al. 1998) and is the first on sexual reproduction on Reunion Island. It was conducted at the Trou d'Eau site, where there is a high fission rate. The site benefits from an upwelling of nutrient-rich water (Cuet 1989), which enables algae to develop and results in high organic-matter content in sediment. These conditions are highly conducive to settlement and proliferation by this species, which is a detritus feeder (Conand 1989; Uthicke 1997).

When using microscopic sex determination, a highly male-biased sex ratio of 0.97:0.03 was observed. This is unusual among other sea cucumber species in which the ratio is often close to 1:1 (Conand 1989; Hopper et al. 1998; Uthicke 1997; Hamel et al. 2001).

Other *S. chloronotus* populations studied in the past have displayed balanced sex ratios, ie in Australia (GBR) (Franklin 1980), Indonesia (Tan Shau-Hwai and Bin Yasin 2000) and Reunion Island (Planch'Alizés site) (Conand unpub. obs.). A comparable sex-ratio bias has, however, been described by Uthicke et al. (1999) on Great Palm Island (GBR). Only one female was observed out of 59 specimens.

There are four possible explanations for these results:

- 1) high adult female mortality;
- 2) a higher fission rate among males;
- 3) low female recruitment owing to high female mortality among larvae or juveniles; and
- 4) sex inversion during the life cycle at the planktonic larval or adult stages.

Weight distribution was identical among males and females, however, indicating that age and survival rates were also equally distributed across both sexes, which would exclude the first hypothesis. No differences in fission rates between the sexes have been observed either (Uthicke et al. 1999), which excludes the second hypothesis.

The fact that size is evenly distributed among both males and females and that no hermaphrodite specimens have been recorded is incompatible with the sex-inversion hypothesis at the adult stage. The heavy sex-ratio bias at Trou d'Eau can therefore be explained either by higher female mortality during recruitment or a differential dispersal ability according to sex, as on the GBR (Uthicke et al. 1999).

The reproductive cycle was described using standard methods. Average monthly GI data can differ depending on the species or site under consideration. The bimodal curve observed with *Stichopus chloronotus* on Reunion Island indicates a biannual reproductive strategy, which confirms previous studies on this species (Franklin 1980; Tan Shau-Hwai and Bin Yasin 2000). There is a significant difference between the peaks' average values (ie statistically significant at a threshold of 5%), which implies different reproductive activity rates at each spawning season. This has been observed before by various authors, namely Franklin (1980), with regard to *S. chloronotus*, as well as by Conand (1989 and 1993b) and Hamel et al. (2001) with regard to *Holothuria scabra*. Mean gonad indices for March 2000 (1.64 ± 0.45) and March 2001 (3.31 ± 1.10) differed significantly ($p = -6.27$ at a 5 % threshold).

Water temperatures in March 2001 exceeded the multi-year average by approximately 1° C. This prolonged warm season brought about a shift and/or extension of the reproductive cycle. This phenomenon has previously been observed by various authors, particularly Hopper et al. (1998), who demonstrated how temperature severely disrupted the reproductive cycle in *Actinopyga mauritiana*.

When values are compared with other authors', several similarities can be observed, such as two reproductive seasons and greater activity in the warm season, but so can a number of differences. Such comparisons can only be made in terms of general reproductive activity trends, however, as each author's selected gonad indices restrict the values than can be compared (total weight, open or eviscerated). The reproductive cycle described in Malaysia (Tan Shau-Hwai and Bin Yasin 2000), for example, appears to extend over a longer period than on Reunion Island. As there is a longer interval between the two reproduction peaks (Fig. 8), the end of the cycle occurs later. Also, an earlier peak has been recorded. The data obtained on the Great Barrier Reef (Franklin 1980) indicates the same interval between peaks as on Reunion Island (Fig. 8). Values are also too small to discern any difference in reproductive activity rates between the two spawning periods. The cycle on the Great Barrier Reef appears to last the same time as on Reunion Island, although the two reproductive cycles seem to occur at slightly different times.

Gonad morphology was examined from two angles: tubule diameter and tubule length. Data on tubule diameters indicated that they were widest during spawning, as observed by Franklin (1980). The longest tubule lengths were recorded during the high reproductive activity period. Tubules were present throughout the year in *S. chloronotus*

and there was no sexual resting phase. Oocyte diameter estimates were obtained from formalin-fixed specimens. An average diameter of 84 μm was obtained and the highest measurement, ie 114 μm , was recorded in the pre-spawning stage. These values are higher than Franklin's who worked on material fixed in Bouin's solution, which causes greater cell retraction than formalin.

The monthly atresia index variations indicated a close link with reproductive activity. The atresia index curve followed a generally opposite path to the GI curve's, with higher values occurring in April and August. This index was useful in corroborating other results.

The histological study confirmed the results obtained on the choice of the various stages using macroscopic observations. Although first maturity was estimated at 50 g in this study on the basis of only 20 specimens, this weight was consistent with Franklin's results (1980).

The proportion of specimens at each stage during the cycle effectively correlated with the GI curve and fairly accurately reflected the specimens' monthly development stages.

The description of *S. chloronotus*'s reproductive cycle was therefore based on an integration of three different parameters, ie 1) variations in GI averages; 2) monthly variations in the average atresia index; and 3) monthly proportions of specimens in each stage.

Conclusions

Stichopus chloronotus's reproduction cycle at the Trou d'Eau, Reunion Island, site does not differ greatly from other populations' as previously studied on Australia's GBR (Franklin 1980) or in Malaysia (Tan Shau-Hwai and Bin Yasin 2000). As on other sites, the cycle covers a year and has two spawning seasons. The Conand et al. (1998) study conducted at the same station nevertheless demonstrated that this population was quite unusual in terms of the high densities recorded (3.7 specimens/ m^2), low average specimen weights and asexual reproduction by fission. It should nevertheless be noted that spawning occurred in the warm season and fission in the cool. This study contributes new information to the quest to understand this population's adaptive strategies and will serve as a basis for assessing the respective roles played by both reproduction methods in population dynamics.

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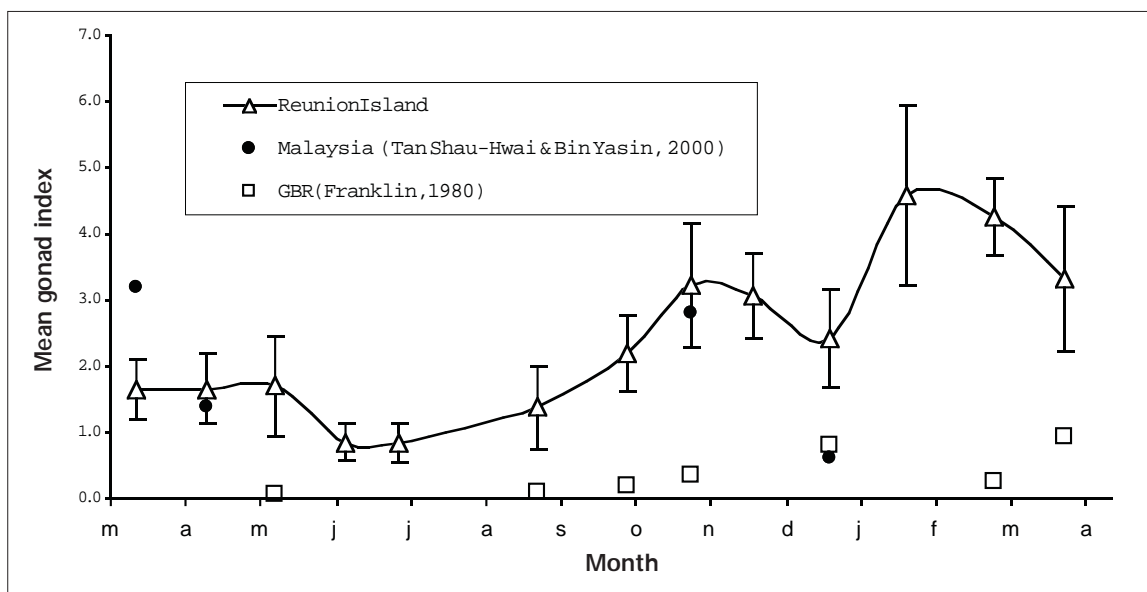


Figure 8. Comparison of the reproductive cycles (Gonad-indices) of *Stichopus chloronotus* between several countries

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Stichopus chloronotus