

Reproductive Cycle of Edible Echinoderms from the South-Western Indian Ocean

II: The sandfish *Holothuria scabra* (Jaëger, 1833)

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Abstract—The reproductive cycle and gonad morphology were investigated in a population of *Holothuria scabra* (Jaëger, 1833) from Toliara, south-west of Madagascar. Surveys were done from November 1998 to April 2001 by monthly samplings of 30 individuals. There is a single gonad formed by a tuft of numerous ramified tubules. The annual reproductive cycle of the population was determined from monthly variations of the gonad index and the maturity index. Five sexual maturity stages were described. An annual reproductive cycle occurred with most individuals being mature or spawning between November and April. Gametogenesis was not synchronous in the population, but was synchronous in all the tubules of a single gonad except the smallest immature ones (length < 1 cm). The length of tubules is not uniform in a gonad, and the number of gonad tubules is related to the size of the animal. Each tubule had a clear annual cycle, being the smallest in recovery gonads (regressed tubules) and the largest at the end of the maturation period (developed tubules). Recruitment of new tubules appears to occur throughout the year. Observations made suggest that the newly recruited tubules remain immature up to the beginning of the next reproductive cycle.

INTRODUCTION

The aspidochirote holothuroid *Holothuria scabra*, also known as the sandfish, is widely distributed in the coastal tropical regions of the whole Indo-West Pacific Ocean (Clark and Rowe 1971; Conand 1998a; and Massin 1999). *H. scabra* is one of the most economically valuable sea cucumber species that is exploited heavily for marketing in Asia. Individuals are harvested mainly in seagrass beds of inner reef flats where they bury themselves in sand during the day and emerge at night. The reproductive cycle of the species has

been studied over most of its geographical range, from the Red Sea to the Philippines and to New Caledonia (e.g. Mortensen 1937; Ong Che and Gomez 1985; Conand 1989; 1993a; see Hamel *et al.* 2002 for review). Various populations showed either an annual, bi-annual or continuous reproductive cycle (Harriot 1980; Conand 1990; 1993a; Morgan 2000; and Ramofafia *et al.* 2003). However, the methods used to investigate reproductive cycles differed markedly from one study to the other, which sometimes renders the comparisons difficult (Hamel *et al.* 2002).

The present work was done in the framework of the settling of a sea-cucumber (*H. scabra*) hatchery and farm near Toliara, south west coast of Madagascar (see Jangoux *et al.*, 2001). *Holothuria scabra* is one of the most exploited species in Toliara region (Rasolofonirina and Conand, 1998) and the resource is now uncommon in the area (Rasolofonirina *et al.*, 2004). Except for a few preliminary observations, no investigation was done on the reproductive biology of the species in the region of the southern Indian Ocean Islands. The present work thus aimed to study, on a monthly basis and over a 30-months period, the reproductive cycle and the external changes of the gonads in a population of *H. scabra* from histological investigations and calculation of individuals' gonad and maturity indices.

MATERIAL AND METHODS

The study was carried out on *H. scabra* individuals collected in seagrass beds of the barrier reef in Toliara bay (Fig. 1). The site is under the influence of a semidiurnal tide of 0.5 to 3 m amplitude. The warm season in Toliara extends from November to April (average sea water temperature in February: 34.3°C) and the cold season from May to October (average sea water temperature in July: 22°C) (Fig. 2). The monsoon rains occurs from December to March (Fig. 2, but rains are rare in Toliara (mean rainfall = 48 cm/year) compared to other regions of Madagascar. The substrate consists of sandy, or sandy-muddy substrates. From November 1998 to April 2001, 30 specimens of *Holothuria scabra* were collected monthly in Toliara bay either by walking at low tides or by free diving. Individuals were transported to the laboratory to be measured, weighed and dissected according to the method of Conand (1989, 1993a). The following measurements were made: the total length to the nearest cm (TL), the total wet weight (TW), the drained weight (DW; weight of the animal after incision and removal of the coelomic fluid), and the body wall weight (BW). A summary of the monthly records and sampling results is presented in Table 1.

The gonad of each individual was weighed and measured (length of longest tubules) then prepared for histological analyses. Gonads were fixed in

Bouin's fluid for 12 h and stored in 70% ethanol. The longest tubules were separated, dehydrated in graded ethanol series, embedded in paraffin, cut into 7 μ m thick sections and stained with Masson Trichrome. Sections were used to determine the sex and maturity stage of each gonad and to measure the diameter of 50 oocytes of each ovary.

For each individual, a gonad index (GI) and a maturity index (MI) were established. The GI was calculated as follows: $GI = G \text{ (wet weight of the gonad)} / BW \text{ (Body wall weight)} \times 100$. The mean monthly GI values were compared using an ANOVA (level of significance: 0.05) followed by a post-hoc Tukey test on the arcsin transformed values (to normalise the data) (Zar, 1996).

The maturity index (MI) was assessed for each sample according to a scale from I to X based on the staging method used for sea urchins by Yoshida (1952) and Spirlet *et al.* (1998). It relies on the number of individual in each maturity stage in the monthly sample and was calculated as follow: $MI = (n_1 \cdot 1 + n_2 \cdot 2 + \dots + n_x \cdot X) / (n_1 + n_2 + \dots + n_x)$, where n_1 to n_x are the number of individuals whose gonads are at maturity stage I to X. The MI thus corresponds to the average maturity stage encountered each month.

An additional batch of 198 individuals was gathered from September 2002 to April 2003 to further investigate gonad anatomy and histology including the number, possible branchings, diameter and length of gonad tubules, and identification of the tubule maturity stage.

RESULTS

Out of the 732 examined individuals, 334 were females, 380 males, 8 of indeterminate sex, and 10 lacked gonads. The Chi-square test on the sex ratio shows no significant difference ($\chi^2 = 3.1$; $P > 0.05$) from 1:1 ratios.

Changes in the gonad index

There was no marked difference between the GI monthly values of male and female individuals and the results were consequently pooled. The average values of the GI are generally low: from less than 0.5% to ca. 5% (Fig. 3). Statistical analyses allowed to note a significant variation of the GI values for

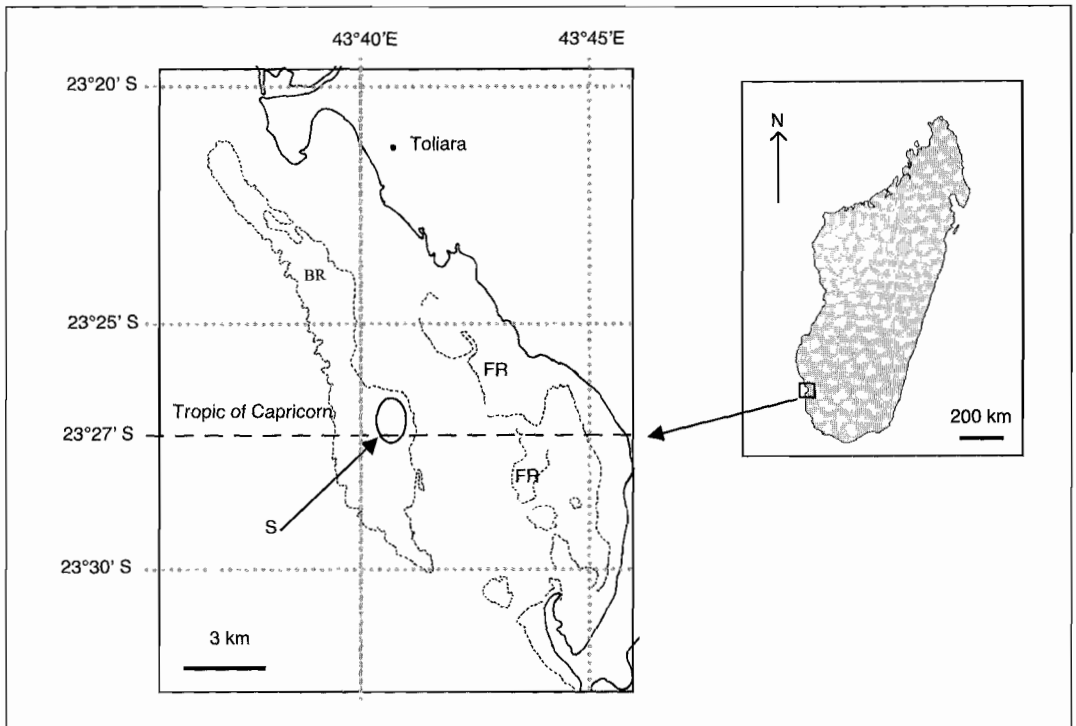


Fig. 1. Maps of Madagascar (right) and of Toliara bay (left) showing the collecting site (S, arrow). BR: Barrier reef; FR: Fringing reef

the year 1999-2000 only. As a general rule, there is a tendency for slightly higher values in the warm season (November to April) in each investigated summer period.

Gonad histology and maturity index

The gonad of *H. scabra* consists in a tuft of numerous tubules of unequal length (Fig. 4A). Most tubules are branched, being often translucent and white (male) to orange (female) in colour. They arise from an inconspicuous though rather dense gonad base where they share a common connective sheet (Fig. 4B). Tubules in the gonad tuft join to each other, and to the gonoduct. The latter is located in the dorsal mesentery, runs parallel to the foregut and opens mid-dorsally to the outside, close to the mouth (Fig. 4C).

Histological observations of the gonads were used to identify gonad stages and calculate individuals' maturity index (MI). Five gonad stages were recognised in the Toliara population of *H.*

scabra (Fig. 5) which were similar to those described by previous authors (e.g. Tanaka 1958; Hamel *et al.* 2002 and Ramofafia *et al.* 2003). These are:

Stage I (spent)

The tubule lumen may still include relict oocytes or spermatozoa together with a few somatic cells (phagocytic cells). No parietal germinal cells are seen but a few scattered oogonia or spermatogonia. In both ovaries and testes, the tubules are translucent to whitish. Their wall appears either retracted or slightly distended (Figs 5A-B).

Stage II (recovery)

There is a thin layer of germinal cells at the periphery of the male tubules measuring up to 9 μm thick, and female tubules may include some small previtellogenic oocytes of 15 to 40 μm in

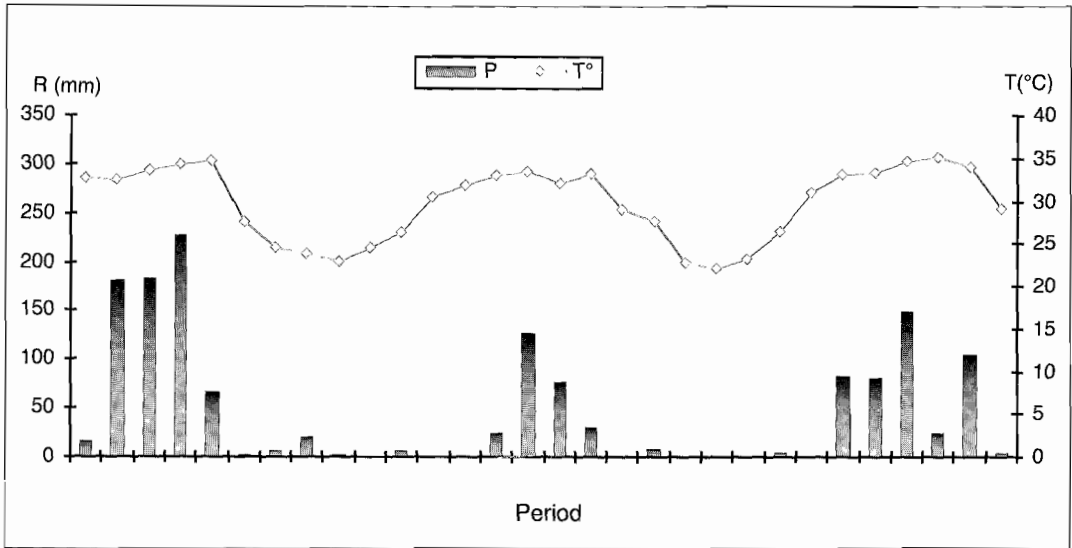


Fig. 2. Monthly variation of sea water temperature (T°) and rainfall (P) in Toliara (November 1998 - April 2001)

Table 1. Summary of monthly records taken from *Holothuria scabra*. T: total number of individuals; F: number of females; M: number of males; ML: mean length; MTW: mean total wet weight; MDW: mean drained weight; MBW: mean body wall wet weight

Month	T	F	M	ML \pm SD	MTW \pm SD	MDW \pm SD	MBW \pm SD
Nov-98	29	16	12	23.8 \pm 2.7	506.2 \pm 107.1	262.4 \pm 92.4	154 \pm 1.6
Dec-98	29	10	19	18.9 \pm 2.0	214.8 \pm 59.2	165 \pm 38.8	118.9 \pm 32.6
Jan-99	27	12	12	22.2 \pm 3	296.3 \pm 128.5	254.4 \pm 60.1	159.8 \pm 44.4
Feb-99	29	16	13	21,3 \pm 1,6	332,0 \pm 81,3	222 \pm 54,5	147,4 \pm 47,4
Mar-99	30	15	13	21,1 \pm 2,3	297,1 \pm 65,8	212,6 \pm 49,5	148,3 \pm 36,9
Apr-99	25	13	11	20,2 \pm 2,9	317,9 \pm 79,1	194,5 \pm 60,5	138,4 \pm 45,5
Mey-99	28	11	16	20,0 \pm 3,3	321,1 \pm 120,8	183,1 \pm 67,6	128,5 \pm 55,7
Jun-99	22	13	8	21,1 \pm 2,4	314,4 \pm 132	179,1 \pm 43,2	127,8 \pm 32,9
Jul-99	25	9	14	20,6 \pm 2,2	328,9 \pm 97,8	240,9 \pm 73,1	164,1 \pm 48,5
Aug-99	26	12	12	19,8 \pm 5,1	286,8 \pm 135,9	214,2 \pm 167,7	169 \pm 131,7
Sept-99	29	16	12	19,4 \pm 3,1	289,0 \pm 121,5	201,2 \pm 80,0	127,2 \pm 66,5
Oct-99	24	11	13	19,5 \pm 2,1	246,8 \pm 105,4	193,5 \pm 72,9	120,6 \pm 52,0
Nov-99	25	11	12	20,8 \pm 2,1	241,2 \pm 77,1	195,7 \pm 56,6	121,1 \pm 37,1
Dec-99	29	8	21	22,3 \pm 2,2	370,7 \pm 114,5	240,7 \pm 69,5	140,3 \pm 46,0
Jan-00	29	16	13	23,2 \pm 2,3	403,1 \pm 78,2	299,5 \pm 60,7	189,3 \pm 48,9
Mar-00	18	3	14			273,2 \pm 67	161,6 \pm 49
Mey-00	24	12	12	22,9 \pm 5	527,8 \pm 226,2	278,4 \pm 84,5	193,1 \pm 46,9
Jun-00	25	13	12		295,2 \pm 77,3	219,3 \pm 58,5	196,4 \pm 67,4
Jul-00	26	13	12	18,9 \pm 3,7	287 \pm 94,6	201,8 \pm 83,9	158,1 \pm 75,2
Aug-00	30	16	14	21,3 \pm 2,3	418,3 \pm 70,3	209,7 \pm 43	130,7 \pm 34,8
Sept-00	29	10	19	18,7 \pm 2,5	308 \pm 104,9	184,3 \pm 44,8	125 \pm 21
Oct-00	27	12	15	16,3 \pm 1,8	218,5 \pm 37,3	156,1 \pm 19,8	122,4 \pm 18,3
Nov-00	27	12	15	15,4 \pm 2,6	177,4 \pm 51,3	130,5 \pm 47,1	108,8 \pm 30,8
Dec-00	17	9	8	14,2 \pm 1,4	168,2 \pm 38,7	107,5 \pm 38,2	93,4 \pm 24
Jan-01	28	12	16	16,5 \pm 3	239,7 \pm 103,9	175 \pm 89,3	148 \pm 76,3
Feb-01	29	11	18	15,9 \pm 2	229,2 \pm 59,9	162,3 \pm 41,9	128,6 \pm 34
Mar-01	30	17	13	16,7 \pm 1,7	229,3 \pm 48,2	163,7 \pm 30,4	137,6 \pm 24,5
Apr-01	21	7	13	14,8 \pm 2,1	261,9 \pm 56,4	191,9 \pm 50	144,5 \pm 41,5

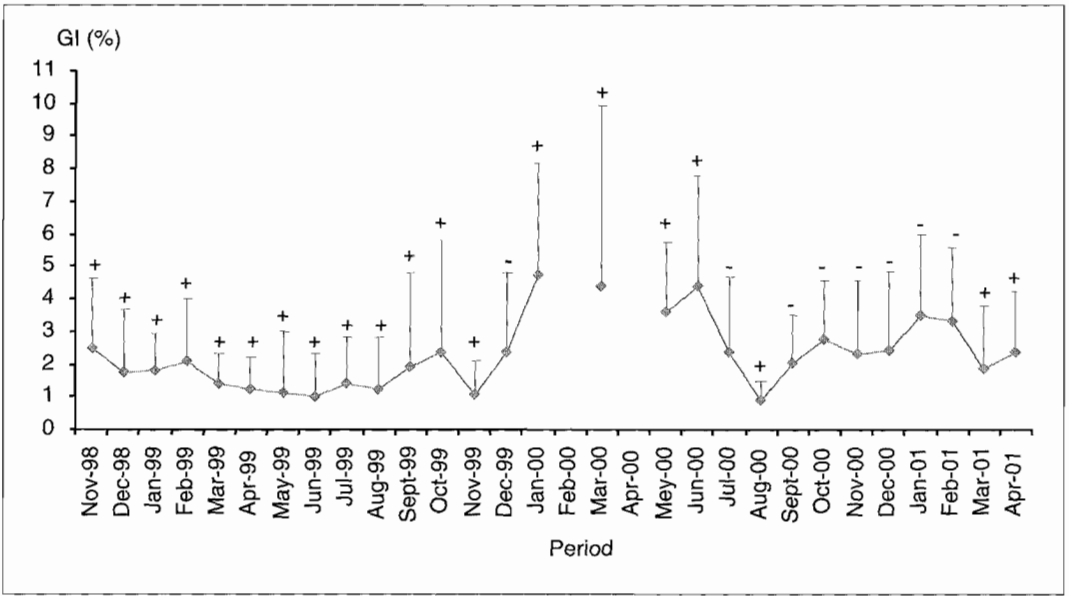


Fig. 3. Change in Gonad Index (GI) for *Holothuria scabra* from November 1998 to April 2001. Mean values and standard deviations (values of n: see Table 1). Two successive different signs (+&- or -&+) means significantly different values (Kruskal-Wallis test; $\alpha = 0,05$)

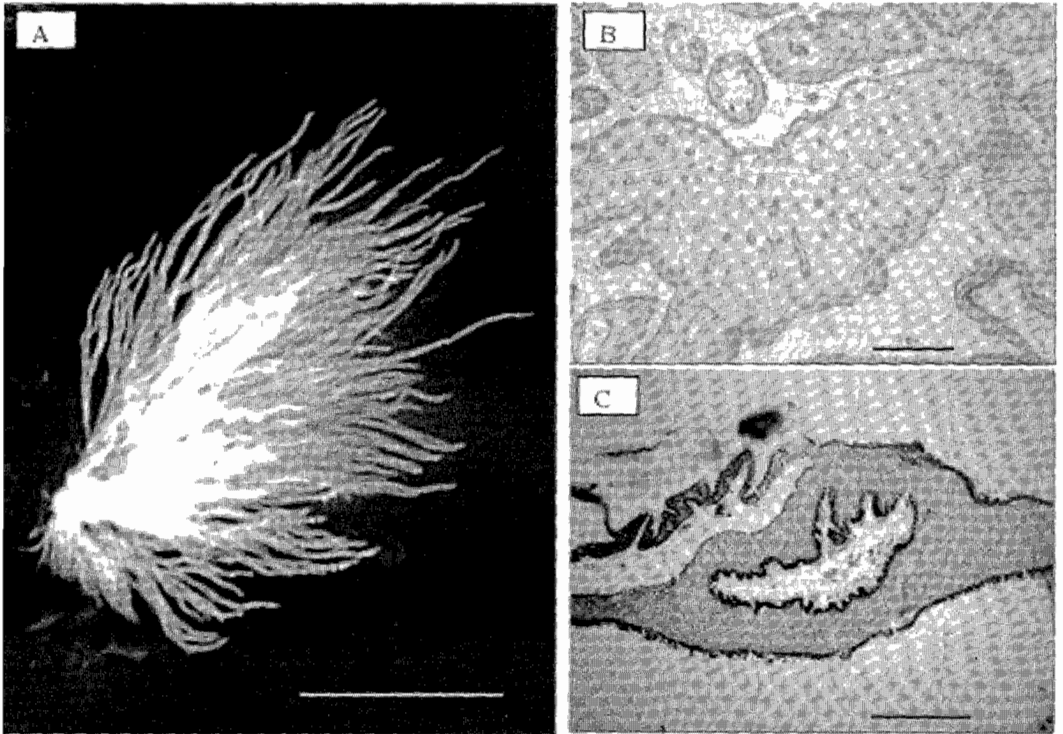


Fig. 4 (A-C). Sections through male *Holothuria scabra* gonads. A. Mature gonad (note the various length of the tubules) (Bar=5cm). B. Section through gonad muff from which the tubules arise (bar=1mm). C. Section through the gonoduct (bar = 0.5 mm)

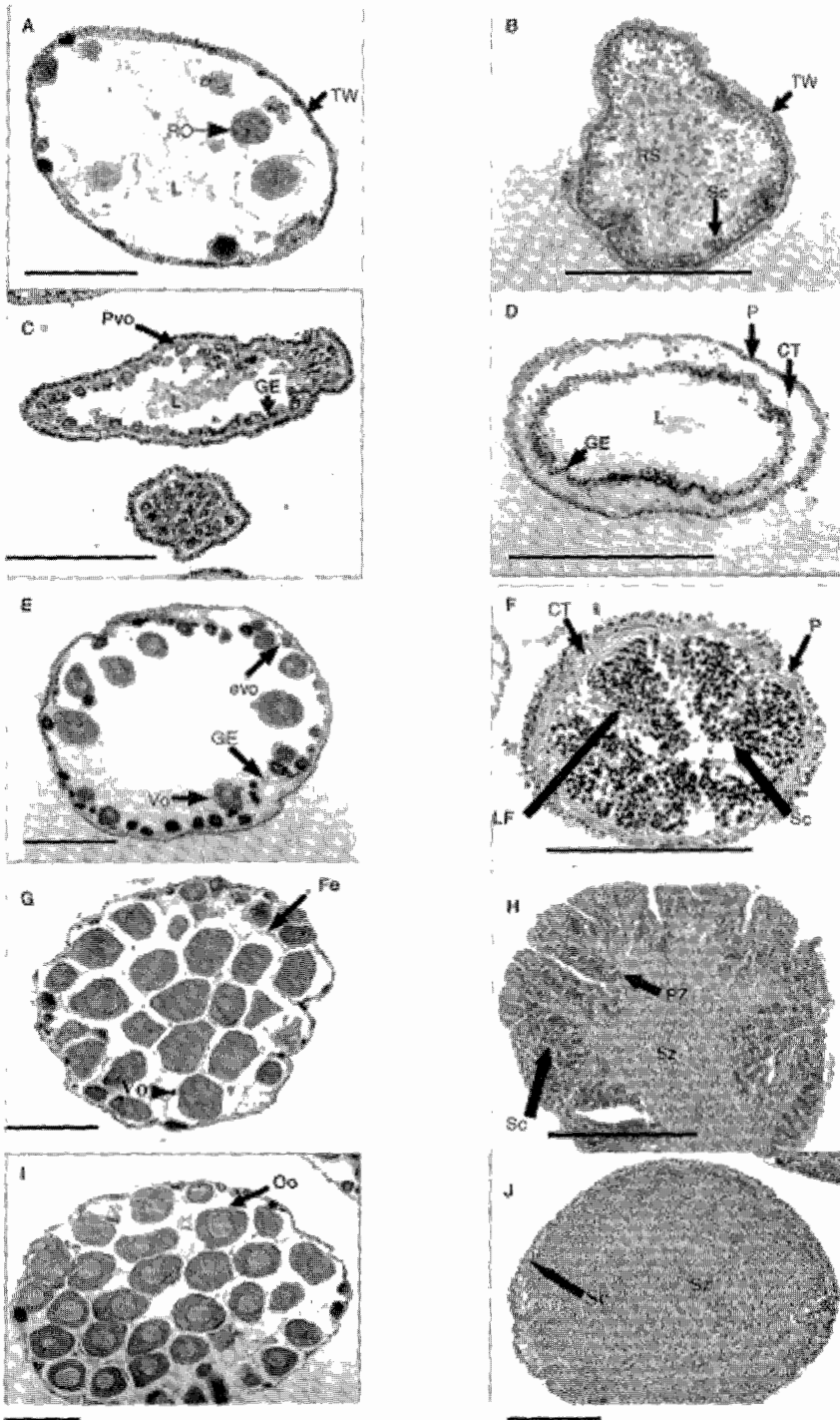


Fig. 5. Gonad stages in female and male *Holothuria scabra*. A, B: Spent (stage 1); C, D: Recovery (stage 2); E, F: Growing (stage 3); G, H: Maturation (stage 4); I, J: Spawning (stage 5). CT: Connective tissue; Evo: early vitellogenic oocyte; Fc: follicular cells; GE: germinal epithelium; L: lumen; LF: longitudinal fold; Oo: oocyte; P: Peritoneum; Pvo: Pre-vitellogenic oocyte; PZ: proliferation zone; RO: relict oocytes; RS: residual spermatozoa; Sp: Spermatocytes; SZ: spermatozoa; TW: tubule wall; Vo: vitellogenic oocyte (Scale bar = 200 μ m)

diameter (Figs 5I-J). Recovery tubules are translucent. Their wall is rather thick and the lumen usually empty (Figs 5C-D).

Stage III (growing)

Corresponds to the beginning of vitellogenesis in ovaries. Oocytes start to grow (from 20 to 120 μm diameter) and progress to the centre of the gonad. Accessory (follicle) cells surround the growing oocytes. In testis, ridges of connective tissue develop towards the centre of the lumen and spermatogonia and spermatocytes are present, the later starting to invade the tubule lumen. An empty space is still visible in the centre of male tubules where a few spermatozoa can be observed. Tubules have a white to yellow colour in testis and ovaries, respectively (Figs 5E-F).

Stage IV (maturation)

There are still a few pre-vitellogenic oocytes close to the ovarian tubule wall. Most oocytes, however, are large vitellogenic oocytes (up to 140 μm) that fill almost completely the tubule lumen being surrounded by rather thin follicular cells. The lumen of male tubules is filled with spermatozoa and spermatocytes, the later still forming a well distinct peripheral layer. Ridges of connective tissue remain at the tubule periphery. The tubules in females are clear to dark orange while in males they are whitish to yellow cream (Figs 5G-H).

Stage V (spawning)

The ovarian tubules are filled with large, rounded oocytes. A few phagocytic cells are locally invading the ovarian tubule. In males, spermatozoa fill most of the tubule lumen, spermatocytes being almost absent. In some sections, remnants of relict spermatocytes and/or spermatozoa can be observed. The tubules in females are orange, while in males they are pale yellow (Figs 5I-J).

The relative frequencies of each gonad stage for female and male gonads are presented in figure 6 (A, B). Gonads at the spawning stage were present almost year round among male individuals, while in females the occurrence of spawning

gonads appears more marked in summer. Based on the relative frequency of each stage (Fig. 6), the maturity index was determined monthly for both males and females. As almost all gonad stages were represented in most of the samples, no clearly distinct cyclical change of the MI was seen from one year to the other (Fig. 7).

Grouping together individuals whose gonads are either at the spawning stage or at the spent stage was the clearest way to demonstrate that such gonads, in both male and female individuals, mostly occur in spring and early summer (Fig. 8).

Another way to tentatively characterise the female's reproductive cycle was to assess the oocytes size frequencies over the investigated period (Fig. 9). Changes in oocytes diameter were not obvious, and individuals with large oocytes (i.e. diameter $>100 \mu\text{m}$) were often seen in the population. Yet small previtellogenic oocytes occurred mostly from May to August (1999) and from May to October (2000), that is from late fall to early spring. Giving that spawning and spent females were mostly seen in summer, this may indicate that female gametogenesis takes about six months to produce mature oocytes.

There was a clear relation between the values of the gonad indices and the gonad stages of individuals, with female gonads tending to gain more weight than male ones and with lighter gonads occurring during recovery (Fig. 10).

The tubules and the reproductive cycle

Tubule size and gonad or body wall weight

The number of tubules per individual clearly increases with both the weight of the gonads (Pearson correlation matrix: $p=0.720$) and that of the body wall of individuals (Pearson correlation matrix: $p=0.713$) (see Fig. 11).

Tubule branchings and tubule length

The gonad in *Holothuria scabra* consists of large branched tubules and small un-branched tubules. The large tubules are mainly two- or three-branched and the proportion of three-branched ones tends to increase with the length of the tubules (Fig. 12).

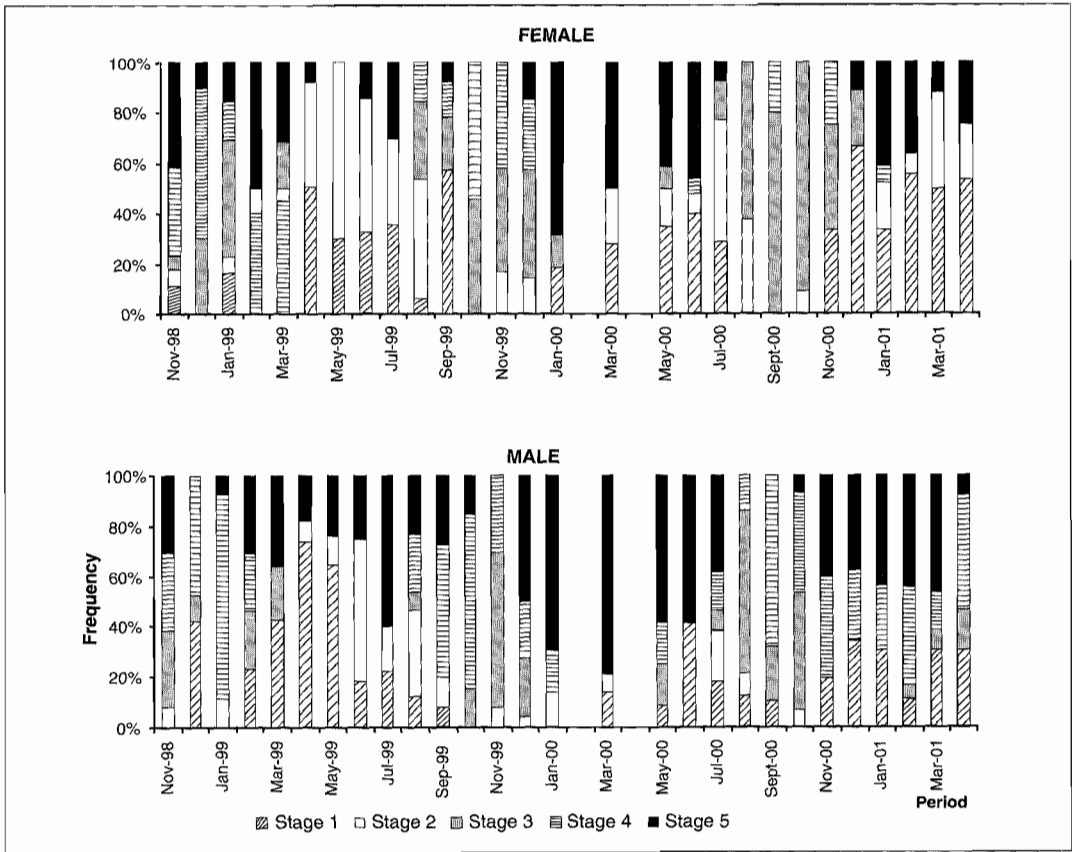


Fig. 6 (A-B). Histogram showing the relative frequencies of each of the five gonad stages for female and male gonads of *Holothuria scabra*

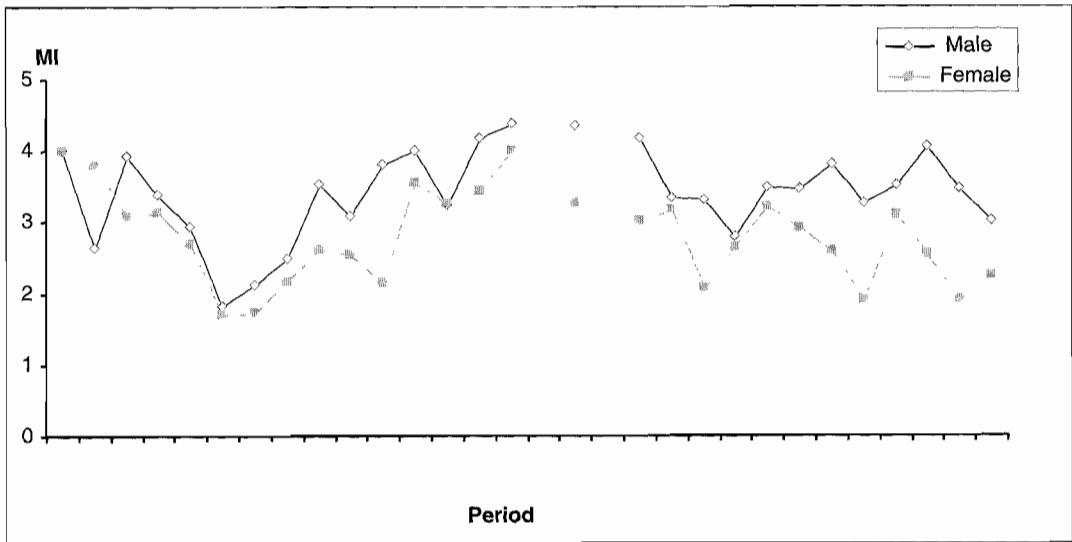


Fig. 7. Monthly change of the Maturity Index (MI) in female and male *Holothuria scabra* individuals from November 1998 to April 2001

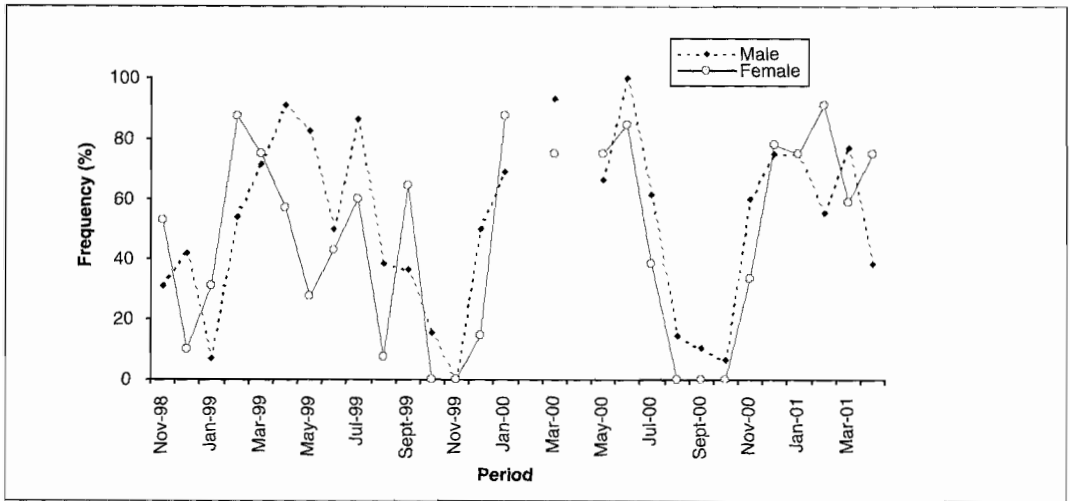


Fig. 8. Relative frequencies (%) of spawning and spent male and female *Holothuria scabra* individuals from November 1998 to April 2001

Tubules and gonad stages

To establish whether all the tubules in the gonad of an individual were at the same maturity stage, histological investigations were performed on 198 different gonads from which 784 tubules were analysed. The sampled tubules covered the whole tubule size range for each investigated gonad. This showed, with the exception of small-sized tubules (length < 1 cm) which were always immature, that all other tubules from a given gonad were at the same stage and developed synchronously.

Similarly, the diameter of the tubules significantly changes with the gonad stage. In this particular case, male and female gonads behave in the same way, showing similar diameter change with the reproductive stage. As for the gonad index, the smallest value for diameters was observed in gonads at the recovery stage (Table 2).

The gonad length (i.e. the length of the longest tubule of the gonads) also changes with the reproductive stage with the shortest tubules being noted in recovering gonads (Table 3). Tubules of developing gonads therefore increase both in length and width.

Whatever the gonad stage and the length of the longest tubule, small immature tubules were always found with a few exceptions (4 individuals over 198). Usually immature tubules were present in relatively high proportions, between 19-67 %

(Table 4). While rather variable in proportion from one gonad to another, immature tubules occur frequently suggesting a constant recruitment process is taking place.

DISCUSSION

The sandfish *Holothuria scabra* is largely distributed between latitudes 30°N and 30°S in the whole tropical Indo-West Pacific region (Hamel *et al.* 2002). From this region, it is also the holothuroid species whose reproduction and reproductive cycle have been most intensively studied (see Table 5). Depending on the population, the reproductive periodicity in *H. scabra* is annual, biannual, triannual or continuous. According to Ramofafia *et al.* (2003) the spawning pattern in the species is either seasonally predictable (high latitudes) or aseasonal (low latitudes). The population in Toliara belongs to the seasonally predictable category and the reproductive periodicity is annual (November to April).

Various environmental factors may influence gametogenesis and time of spawning (Hamel *et al.* 2002). In Toliara, temperature appears to be involved in synchronising gonad maturity in female *H. scabra*, though it does not appear to affect gonad maturity in male individuals. Other factors such as daylength or salinity also may

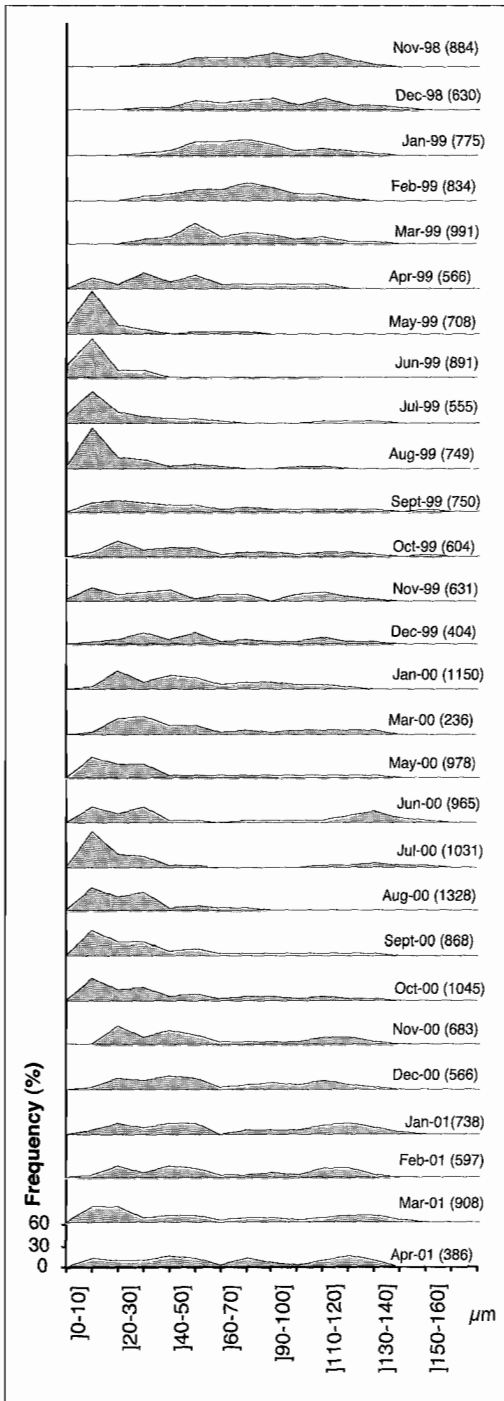


Fig. 9. Frequency distribution of oocyte diameters (in μm) in gonads of all female *Holothuria scabra* sampled from November 1998 to April 2001. Note: 50 nucleolate oocytes from each ovary were measured; the total number of measured oocytes (n) for each month is given in parenthesis

influence reproduction in *H. scabra* (the warmer period in Toliara is also the rainy period which means a decrease in salinity in the lagoon).

Though synchronisation in gonad development is little marked at the population level, it is obvious at the individual level - the development of the constitutive tubules in each gonad is synchronous (Conand, 1993a; Ramofafia *et al.*, 2003; present study). Indeed, all tubules except the smallest ones (length $< 1\text{cm}$), were found to be at the same maturity stage. Clearly *H. scabra* does not fit with the tubule recruitment growth model of Smiley (1988) where various cohorts of tubules are progressively recruited (see also Engstrom, 1980; Tuwo and Conand, 1992). On the contrary, *H. scabra* gonads behave as already reported in several tropical holothuroid species whose tubules develop synchronously (Sewell *et al.*, 1997; Ramofafia *et al.*, 2000, 2003). Tubule recruitment in *H. scabra* takes place as their number increases with gonad and body wall weight, and small immature tubules are always present whatever the size and developmental stage of the gonad. The precise way in which new tubules are recruited in *H. scabra* remains unresolved, especially with regard to their high variability in number (see Table 4). The results suggest that new tubules are not recruited once a year, but rather are recruited year round with a high variability among individuals. However, it was not possible to determine if small immature tubules in a given gonad regularly increase in number in the course of the reproductive cycle. However, if new tubules do not grow or mature when they are recruited in the course of a gametogenetic cycle, they may develop at the onset of the next reproductive cycle as reported for the temperate species *Psolus fabricii* (see Hamel *et al.*, 1993).

Large active tubules in Toliara sandfish clearly have an annual gametogenetic cycle with tubules visible yearlong regardless of the size of individuals. In contrast, Kithakeni and Ndaro (2002) reported the absence of gonads in up to 50% of the *H. scabra* individuals collected in some summer months in Tanzania. In Toliara, a well-marked recovery (resting) period was seen, during which gonad tubules regress in length and diameter. The mean value of tubule diameter

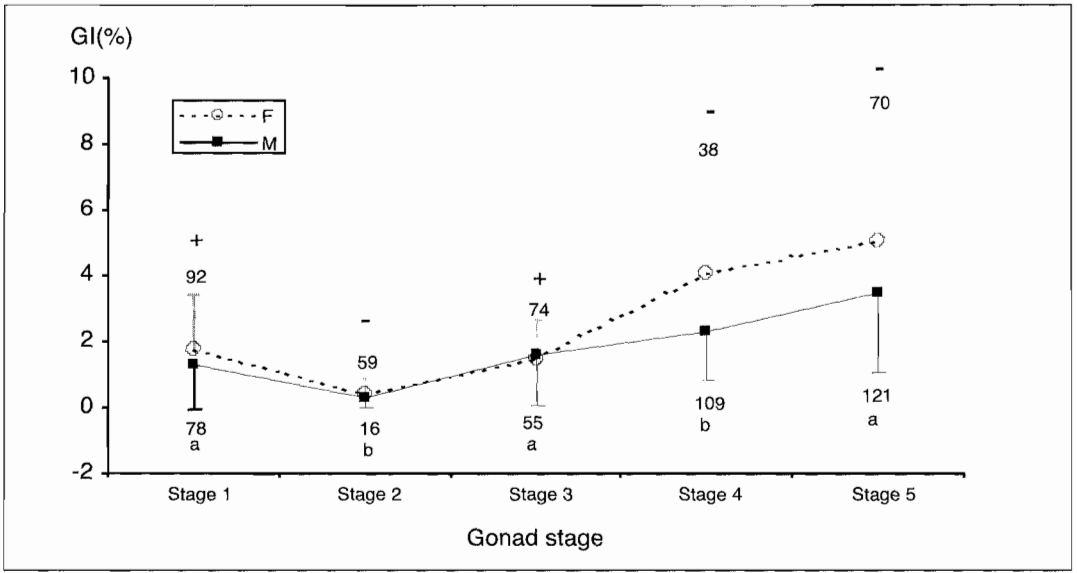


Fig. 10. Relationship between gonad index (GI) and gonad stages 1-5 for *Holothuria scabra* females (F) and males (M) (see also Figs 5 and 6). When two successive but different signs ('+' followed by '-', or vice versa) are given, the values are significantly different (Kruskal-Wallis test; $\alpha = 0,05$). Sample size 'n' for the number of gonads weighed, is given as the figure at the end of the error bars, and varies from 16 to 121)

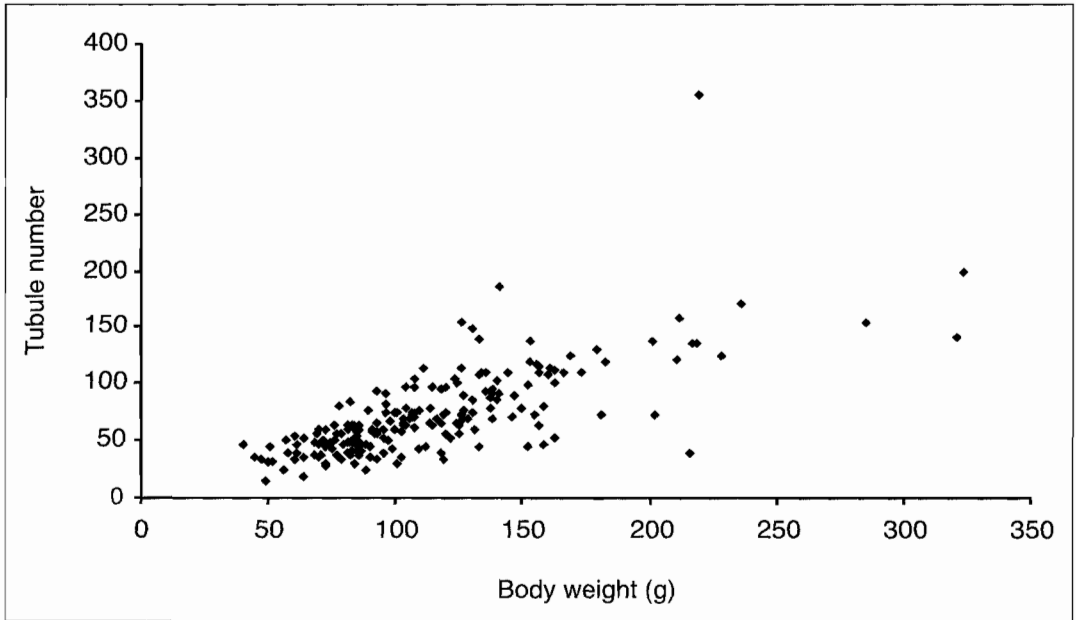


Fig. 11. Relationship between tubule number and body wall weight for *Holothuria scabra* (male and female individuals pooled). (n= 204 gonads)

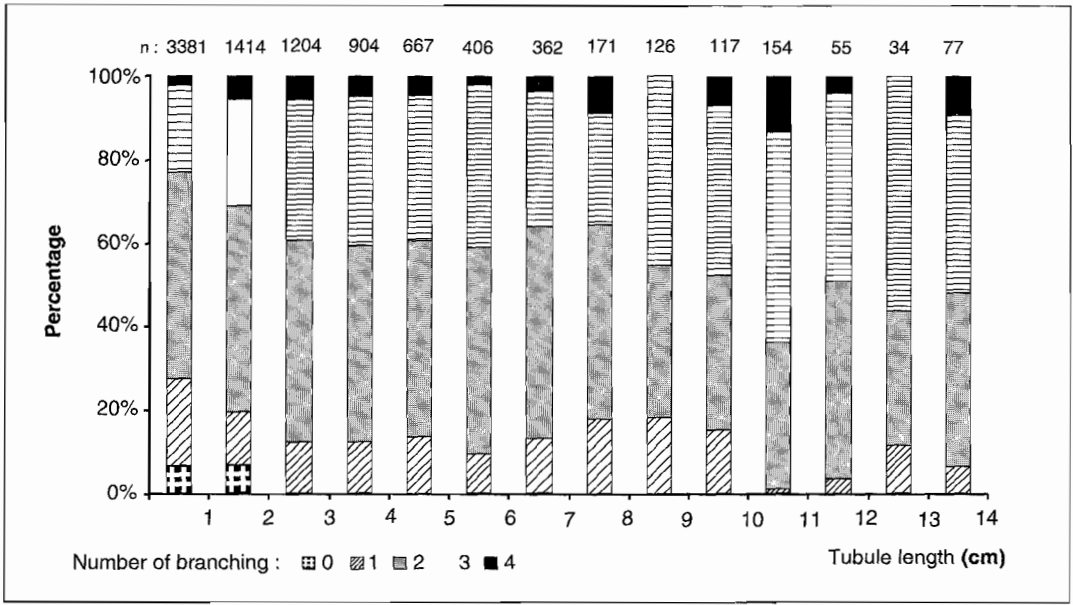


Fig. 12. Frequency of tubule branchings of *Holothuria scabra* with respect to length (n: total number of observed tubules, from 198 gonads examined)

Table 2. Tubule diameter (μm) and gonad stage in female and male individual *Holothuria scabra* (M= mean value; SD= standard deviation ; n= number of observed gonads)

Gonad stage	Tubule diameter (Female)			Tubule diameter (Male)		
	M	SD	n	M	SD	n
Stage 1	654	148.6	92	460	92.3	78
Stage 2	385	90.5	59	314	49.9	16
Stage 3	683	149.8	74	582	121.2	55
Stage 4	860	166.5	38	774	167.4	109
Stage 5	869	157.4	69	822	199.3	121

Table 3. Gonad length (cm) and gonad stage in female and male individual *Holothuria scabra* (M= mean value ; SD= standard deviation; n= number of observed gonads)

Gonad stage	Gonad length (Female)			Gonad length (Male)		
	M	SD	n	M	SD	n
Stage 1	6.1	2.6	91	6	3.2	78
Stage 2	3.6	2.0	59	3.4	1.5	16
Stage 3	5.8	3.0	74	6.5	3.4	54
Stage 4	11	4.2	38	7.2	2.7	109
Stage 5	9.5	3.5	70	8.6	3.7	119

Table 4. Proportions of small (< 1 cm) immature tubules (ranges and mean values) in *Holothuria scabra* for all gonad stages and each sex

Gonad stage and sex	Number of investigated gonads	Proportions of small immature tubules			
		Range (%)	Mean (%)	S D	
1 (spent)	male	17	34.0 to 100	42.7	30.9
	female	12	20.9 to 62.5	36.8	13.6
2 (recovery)	male	8	31.9 to 100	67.0	21.9
	female	11	21.9 to 88.6	54.3	21.9
3 (growing)	male	19	10.5 to 72.7	36.3	13.6
	female	21	1.6 to 74.5	29.4	16.6
4 (maturation)	male	47	0.0 to 68.1	22.5	15.6
	female	11	0.0 to 36.3	19.5	13.8
5 (spawning)	male	28	0.0 to 86.8	21.5	22
	female	22	3.3 to 61.2	27.5	15.3

Table 5. Reproduction in *Holothuria scabra* populations from different locations, using a range of methods to determine the reproductive cycle (GI: Gonad Index; MI: Maturation Index; MMF: Macroscopic and Microscopic Features)

Latitude range	Location	Methods used	Length of the study (months)	Reproductive cycle	Length of periods of maturation + spawning	References
05°S	Sulawesi, Indonesia	GI and histology	12	Biannual	8 months	Tuwo, 1999
08°S	Solomon Island, Pacific Ocean	GI and histology	30	Continuous	12 months	Ramofafia <i>et al.</i> , 2003
09°N	Gulf of Mannar, India.	GI	12	Biannual.	No data	Krishnaswamy and Krishnan, 1967
13°N	Calatagan, Phillipines	GI	12	Triannual	No data	Cowan and Gomez, 1982
13°N	Calatagan, Phillipines	GI and histology	24	Continuous	12 months	Ong Che and Gomez, 1985
22°S	New Caledonia	GI & MMF	20	Biannual	6 months	Conand, 1990
23° S	Toliara, Madagascar	GI, MI and histology	30	Annual	6 months	Present study
27°S	Moreton bay, Australia	GI and histology	16	Annual	2 months	Morgan, 2000

changes from 800 to 400 μm , and that of tubule length from almost 10 to less than 4 cm. Such regression process did not occur in the Salomon population of *H. scabra* (Ramofafia *et al.* 2003).

Compared to *H. scabra* from other sites (Baird 1974; Conand, 1989, 1998b), it appears that the average sizes of the individuals from Toliara are small (see Table 1). Though local conditions may influence individual size (e.g., Ong Che 1990; Conand 1993b), it should be noted that *H. scabra* is overexploited in Madagascar (Mara *et al.* 1998; Rasolofonirina *et al.* 2004) which could have an

impact on the individual size (Conand *et al.* 1997, Conand *et al.* 1998). If the fishery is not managed in a new future, the only possibility to maintain this valuable economical resource is through hatchery and a farm complexes for sandfish cultivation.

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REFERENCES

- Baird, R.H. (1974). Beche-de-mer of the South Pacific Islands: A handbook for fishermen. South Pacific Commission, Noumea, New Caledonia.
- Clark, A.M. & Rowe, F.W.E. (1971). Monograph of shallow-water Indo-west Pacific Echinoderms. Trustees of the British Museum, London. 238pp. + 30 pls.
- Conand, C. (1998a) Holothurians. In FAO species identification guide. The marine living resources of the Western Central Pacific. vol 2: Cephalopods, crustaceans, holothurians and sharks. K.E. Carpenter & V. Niem, eds. Pp. 1157-1190. FAO, Roma.
- Conand, C. (1998b). Overexploitation in the present world sea cucumber fisheries and perspectives in mariculture. In: R. Mooi and M. Telford (eds). Echinoderms: San Francisco. Rotterdam: A.A. Balkema Press. Pp. 449-454.
- Conand, C. (1993 a). Reproductive biology of the characteristic holothurians from the major communities of the New Caledonia lagoon. *Mar. Biol.* **116**: 439-450.
- Conand, C. (1993 b). Ecology and reproductive biology of *Stichopus variegatus* an Indo-Pacific coral reef sea cucumber (Echinodermata: Holothuroidea). *Bull. Mar. Sci.* **52**: 970-981.
- Conand, C. (1990). The fishery resources of Pacific Island countries. Part 2: Holothurians. *FAO Fish. Techn. Pap.* **272**: 143pp.
- Conand, C. (1989). Les holothuries aspidochirotés du lagon de la Nouvelle-Calédonie : biologie, écologie et exploitation . Etudes et Thèses, ORSTOM, Paris. 393pp.
- Conand, C., De San, M., Refeno, G., Razafitseheno, G., Mara, E. & Andrianjatovo, S. (1998). Difficulties and Sustainable management of the sea cucumber fishery sector in Madagascar. *Bêche-de-mer Bull.* **10**: 7-9.
- Conand, C., Galet Lalande, N., Randriamiarana, H., Razafitseheno, G. & De San, M. (1997). Sea cucumbers in Madagascar: Difficulties in the fishery and sustainable management. *Bêche-de-mer Bull.* **9**: 4-5.
- Cowan, M.E. & Gomez, E.D. (1982). A preliminary note on the reproductive periodicity of the sea cucumber *Holothuria scabra*. *Philippine J. Biol.* **11**: 175-178.
- Engstrom, N.A., (1980). Reproductive cycles of *Holothuria (Halodeima) floridana*, H. (H.) *mexicana* and their hybrids (Echinodermata: Holothuroidea) in southern Florida, USA. *Intern. J. Invertebr. Reprod.* **2**: 237-244.
- Hamel, J.F., Conand, C., Pawson, D.L. & Mercier, A. (2002). The sea cucumber *Holothuria scabra* (Holothuroidea: Echinodermata): Its biology and exploitation as Beche-de-mer. *Adv. Mar. Biol.* **41**: 129-223.
- Hamel, J.F., Himmelman, J.H. & Dufresne, L. (1993). Gametogenesis and spawning of the sea cucumber *Psolus fabricii* (Duben and Koren). *Biol. Bull.* **184**: 125-143.
- Harriott, V.J. (1980). The ecology of holothurian fauna of Heron Reef and Morton Bay. MSc thesis, University of Queensland, Brisbane, Australia. 153pp.
- Jangoux, M., Rasolofonirina, R., Vaitilingon, D., Ouin, J.M., Seghers, G., Mara, E., and Conand, C. (2001). A sea cucumber hatchery and mariculture project in Toliara, Madagascar. *Bêche-de-mer Bull.*, **14**: 2-5.
- Kithakeni, T. & Ndaró, S.G.M. (2002). Some aspects of sea cucumber, *Holothuria scabra* (Jaeger, 1935), along the coast of Dar es Salaam. *Western Indian Ocean J. Mar. Sci.* **1**: 163-168.
- Krishnaswamy, S. & Krishnan, S. (1967). A report on the reproductive cycle of the holothurian, *Holothuria scabra* Jaeger. *Current Sci.* **36**: 155-156.
- Mara, E., Rasolofonirina, R., Rabesandratana, H., Rakotoarinivo, W., Ravelo, I. & Conand, C. (1998). Etude de la pêche aux holothuries et propositions de mesures d'aménagement dans le Sud-ouest de Madagascar. Report IH.SM/ONE/BM, Toliara. 40pp.
- Massin, C. (1999). Reef-dwelling Holothuroidea (Echinodermata) of the Spermonde archipelago (south-west Sulawesi, Indonesia). *Zoo. Verh. Leiden* **329**: 1-144.
- Morgan, A.D. (2000). Aspects of the reproductive cycle of the sea cucumber *Holothuria scabra* (Echinodermata: Holothuroidea). *Bull. Mar. Sci.* **66**: 47-57.
- Mortensen, T.H. (1937). Development and Larval Forms of Echinoderms. III. *Kong. Danske Vidensk. Selsk. Skr.* (7) **9** (1): 1-65 + 15 pls.
- Ong Che, R.G. (1990). Reproductive cycle of *Holothuria leucospilota* Brandt (Echinodermata: Holothuroidea) in Hong Kong and the role of body

- tissues in reproduction. *Asian Mar. Biol.* **7**: 115-132.
- Ong Che, R.G. & Gomez, E.D. (1985). Reproductive periodicity of *Holothuria scabra* Jaeger at Calatagan, Batangas, Philippines. *Asian Mar. Biol.* **2**: 21-30.
- Ramofafia, C., Battaglione, C.S., Bell, J.D. & Byrne, M. (2000). Reproductive biology of the commercial sea cucumber *Holothuria fuscogilva* in the Solomon Islands. *Mar. Biol.* **136**: 1045-1056
- Ramofafia, C., Byrne, M. & Battaglione, S.C. (2003). Reproduction of the commercial sea cucumber *Holothuria scabra* (Echinodermata: Holothuroidea) in the Solomon Islands. *Mar. Biol.* **142**: 281-288.
- Rasolofonirina, R. & Conand, C. (1998). Sea cucumber exploitation in Toliara region, south-west of Madagascar. *Bêche-de-mer Bull.* **10**: 10-15.
- Rasolofonirina, R., Mara, E. and Jangoux, M. (2004). Sea cucumber fishery and mariculture in Madagascar: a case study of Toliara, South-west of Madagascar :133-149 in Lovatelli, C. Conand, S. Purcell, S. Uthicke, J-F.Hamel & A. Mercier (eds.) Advances in sea cucumber aquaculture and management. FAO Fisheries Technical Paper No. 463, 425 pp.
- Sewell, M.A., Tyler, P.A., Young, C.M. & Conand, C. (1997). Ovarian development in the class Holothuroidea: a reassessment of the "tubule recruitment model". *Biol. Bull.* **192**: 17-26.
- Smiley, S. (1988). The dynamics of oogenesis and the annual ovarian cycle of *Stichopus californicus* (Echinodermata: Holothuroidea). *Biol. Bull.* **175**: 79-93.
- Spirlet, C., Grosjean, P. & Jangoux, M. (1998). Reproductive cycle of the echinoid *Paracentrotus lividus*: analysis by means of the maturity index. *Invert. Reprod. Devel.* **34**: 69-81.
- Tanaka, Y. (1958). Seasonal changes occurring in the gonad of *Stichopus japonicus*. *Bull. Fac. Fish. Hokkaido Univ.* **9**: 29-36.
- Tuwo, A. (1999). Reproductive cycle of the holothurian *Holothuria scabra* in Saugi Island, Spermonde Archipelago, Southwest Sulawesi, Indonesia. *Bêche-de-mer Bull.* **11**: 9-12.
- Tuwo, A. & Conand, C. (1992). Reproductive biology of the holothurian *Holothuria forskali* (Echinodermata). *J. Mar. Biol. Ass. U.K.* **72**: 745-758.
- Yoshida, M. (1952). Some observations on the maturation of the sea urchin *Diadema setosum*. *Annotes Zool. Jap.* **25**: 265-271.
- Zar, J.H. (1996). Biostatistical Analysis (3rd ed.). Prentice-Hall. cop., New York.