



## SEXUAL CYCLE OF THREE COMMERCIALY IMPORTANT HOLOTHURIAN SPECIES (ECHINODERMATA) FROM THE LAGOON OF NEW CALEDONIA

Chantal Conand

### ABSTRACT

The population and reproductive biology of three commercially important holothurian species, *Thelenota ananas*, *Microthele nobilis* and *Microthele fuscogilva*, were studied from the lagoon near Noumea, New Caledonia. Samples were taken at approximate monthly intervals by free or scuba diving at certain set points. By examining the morphology and microscopic anatomy of the gonads, a five-stage maturity scale was defined. Simultaneous observation of variations of the gonad index, percentages of different maturity stages and individuals of undetermined sex, made it possible to define the reproductive cycle of these species. The time of gonad growth, maturation, spawning, post-spawning and resting have been related to the annual sea temperature cycle. *Thelenota ananas* and *Microthele fuscogilva* reproduce in the warm season, whereas *M. nobilis* reproduces during the cold season. The fecundity of *M. nobilis* is higher than that of the other two species. The length and weight at first sexual maturity have been defined.

Certain species of holothurians (Echinodermata) are harvested in the shallow waters of the Indo-West Pacific region. They are processed into trepang or bêche-de-mer, a delicacy amongst some oriental populations. This skilled activity was practiced in New Caledonia during the nineteenth and at the beginning of the twentieth centuries and is becoming popular once more (Conand, 1979). Similar renewed interest is also evident in other countries (Sachithanathan, 1972; Motet, 1976; Gentle, 1979; Anonymous, 1979).

Despite their abundance in coral reef communities, little is known about the biology and ecology of holothurians and most information is found in taxonomic and faunal studies (Bakus, 1973). With the intention of management of this resource, population dynamics of the principal commercially exploitable species should be studied. Approximately 10 species may be harvested on the coral-reef complex of New Caledonia; they can be classified according to the commercial value of the processed products. Three species produce a high quality product: the prickly redfish, *Thelenota ananas* (Jaeger, 1883) and the black and white forms of the teatfish, *Holothuria (Microthele) nobilis* (Selenka, 1867) and *Holothuria (Microthele) fuscogilva* (Cherbonnier, 1980), (Fig. 1).

*T. ananas*, belonging to the Stichopodidae family, is widely distributed in the tropical Indo-West Pacific region (Mitsukuri, 1912; Clark and Rowe, 1971). In former times it was extremely popular for the preparation of bêche-de-mer (Clark, 1921; Panning, 1944).

The two forms of the teatfish are clearly distinguished by fishermen from certain Pacific islands (Fiji, Kiribati, Papua New Guinea) who call them by different names. The black form is evenly colored except in the case of small individuals which have cream or orange colored spots (Fig. 1). The white form varies in color from brown to gray or cream, roughly spotted with brown. The black form has cuvierian tubules. Apart from these features, the structure of the anal papillae and differences in the morphology of the spicules have made it possible to distinguish between the black form, *Microthele nobilis*, and the white form, *Microthele fuscogilva* (Cherbonnier, 1980).

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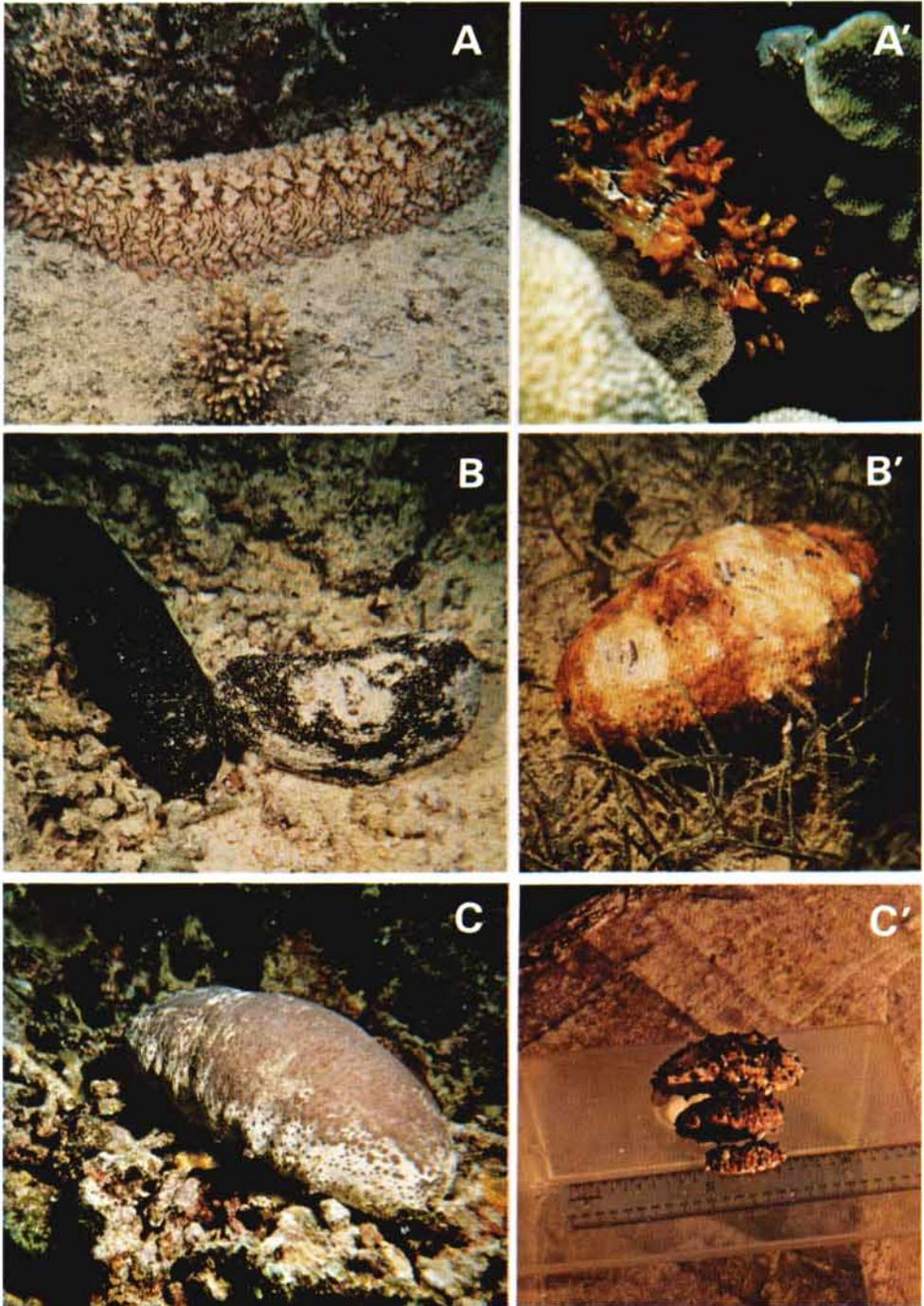


Figure 1. Some holothurian species of commercial interest: A, *Thelenota ananas*, adult; A', *T. ananas*, young specimen; B, *Microthele nobilis*, adult; B', *M. nobilis*, young form; C, *Microthele fuscogilva*, adult; C', *M. fuscogilva*, young form (from Fiji).

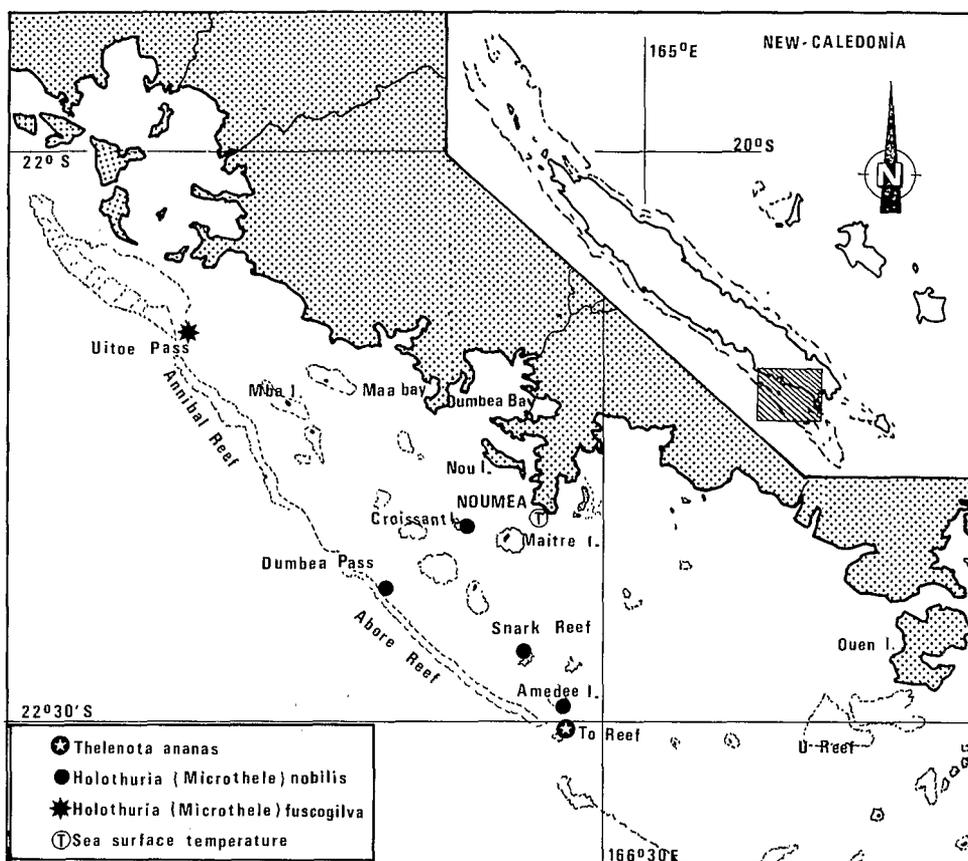


Figure 2. Sampling sites.

These species were studied to define the characteristics of their populations and their reproductive biology. Data published on the reproductive habits of aspidochirote holothurians show wide variations between species and localities (Booolootian, 1966); the few studies already undertaken in tropical waters describe diverse reproductive cycles (Krishnaswamy and Krishnan, 1967; Pearse, 1968; Harriot, 1980).<sup>1</sup>

## METHODS

Some aspects of the distribution of the abovementioned species have already been studied (Intes and Menou, 1979), resulting in the selection of zones of abundance. These species were collected from lagoon sites near Noumea (Fig. 2) at approximate monthly intervals from October 1978 until June 1980, except for the white teatfish which was collected every 2 months. *Thelenota ananas* was sampled at To Reef site, *Microthele nobilis* at the Amédée lighthouse, Croissant Islet and the Aboré and Snark reefs and *Microthele fuscogilva* at the Uitoé pass.

Individuals were generally scattered, making density assessment by quadrat or transect difficult. Indirect density measurements could be obtained during the dive by assessing the Catch Per Unit Effort (CPUE). The unit adopted was the number of individuals collected per diver per hour.

<sup>1</sup> The ecology of holothurian fauna of Heron Reef and Moreton Bay. M.Sc. thesis, University of Queensland, unpublished.

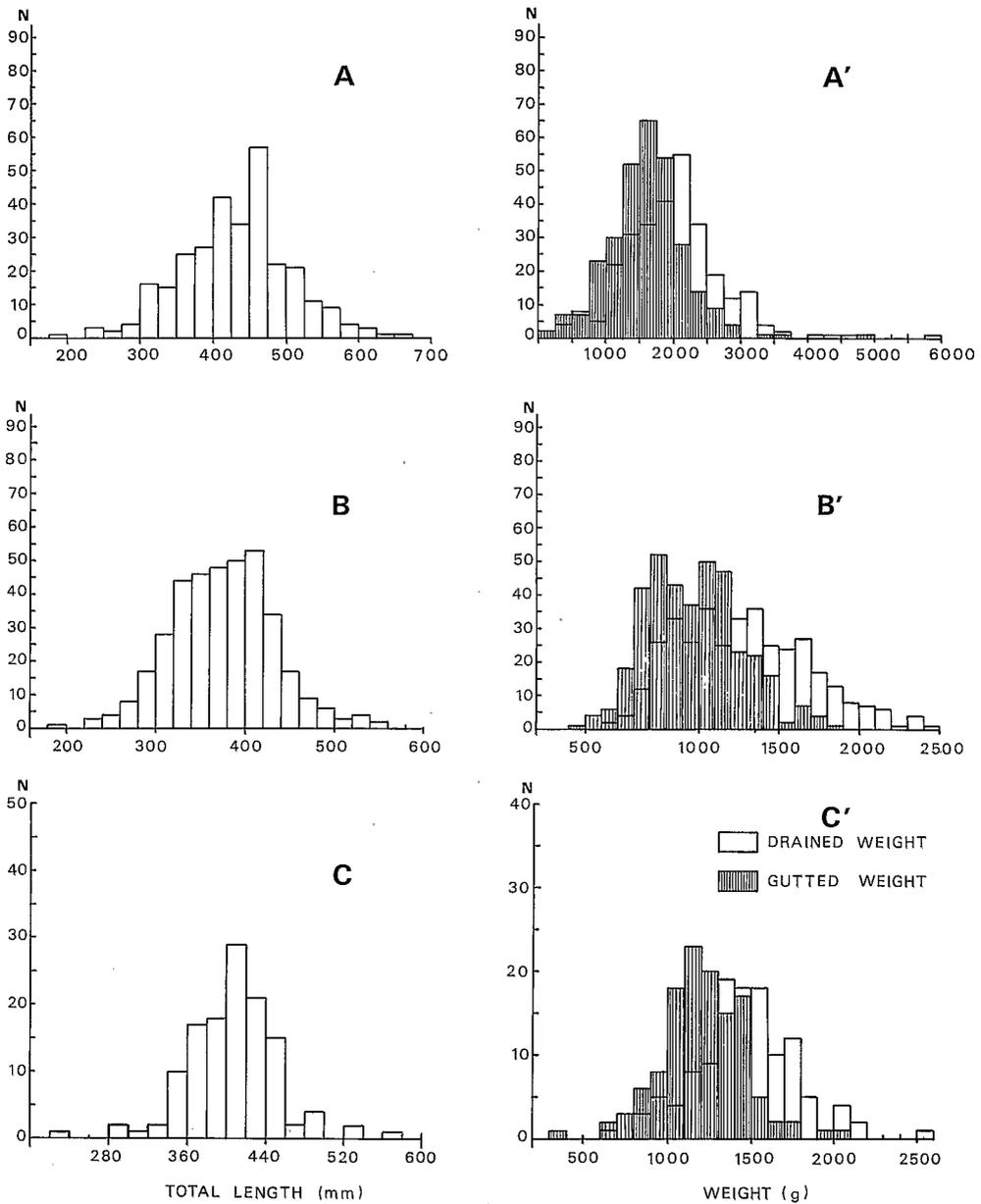


Figure 3. Biological characters in a population of *T. ananas*: A, Total length; A', Weights. *M. nobilis*: B, Total length; B', Weights. *M. fuscogilva*: C, Total length; C', Weights.

Roughly 20 specimens were sampled during each skin or scuba dive. They were transported to the laboratory in sea-water tanks containing magnesium chloride to relax them and were kept overnight in cold storage at a temperature of 4°C. The sample was examined on the following day, recording: total length (TL) to the nearest 5 mm, measured dorsally from mouth to anus by means of flexible ruler; total weight (TW) to the nearest 5 g; drained weight (DW) to the nearest 5 g following the opening of the body and the removal of coeliac water; gonad weight (G) to the nearest 0.1 g; gutted weight (GW) to the nearest 5 g following removal of gonads, and alimentary canal and respiratory trees.

Table 1. Frequency distribution of measured characters (n = sample size, R = range of the values, m = mean, SD = standard deviation)

	<i>Thelenota ananas</i>				<i>Microthele nobilis</i>				<i>Microthele fuscogilva</i>			
	n	R	m	SD	n	R	m	SD	n	R	m	SD
TL (mm)	298	180-670	430	75	377	190-550	370	58	125	230-570	401	46
TW (g)	265	220-6,250	2,180	854	373	390-4,550	1,878	654	108	1,005-3,600	2,111	530
DW (g)	290	205-5,850	1,979	723	367	275-2,410	1,269	409	120	685-2,590	1,464	308
GW (g)	298	175-4,800	1,600	565	375	235-1,875	963	290	124	320-2,000	1,209	253

Table 2. Relations between characters (i = confidence interval at 0.95 level)

x	y	<i>Thelenota ananas</i>				<i>Microthele nobilis</i>				<i>Microthele fuscogilva</i>			
		d.f.	r	Equation	i	d.f.	r	Equation	i	d.f.	r	Equation	i
(1) TL	DW	288	0.84	$y = 230.52e^{0.0048x}$	0.0045-0.0051	363	0.69	$y = -1,451.86 + 7.34x$	6.81-7.87	118	0.67	$y = -1,366.1 + 700x$	600-800
(2) GW	DW	288	0.98	$y = -74.59 + 1.28x$	1.25-1.31	365	0.96	$y = -87.69 + 1.41x$	1.37-1.45	118	0.97	$y = -96.61 + 1.28x$	1.23-1.33
(3) TW	DW	259	0.97	$y = 92.79 + 0.86x$	0.83-0.89	363	0.82	$y = 94.79 + 0.62x$	0.59-0.65	104	0.78	$y = 292.74 + 0.43x$	0.38-0.48

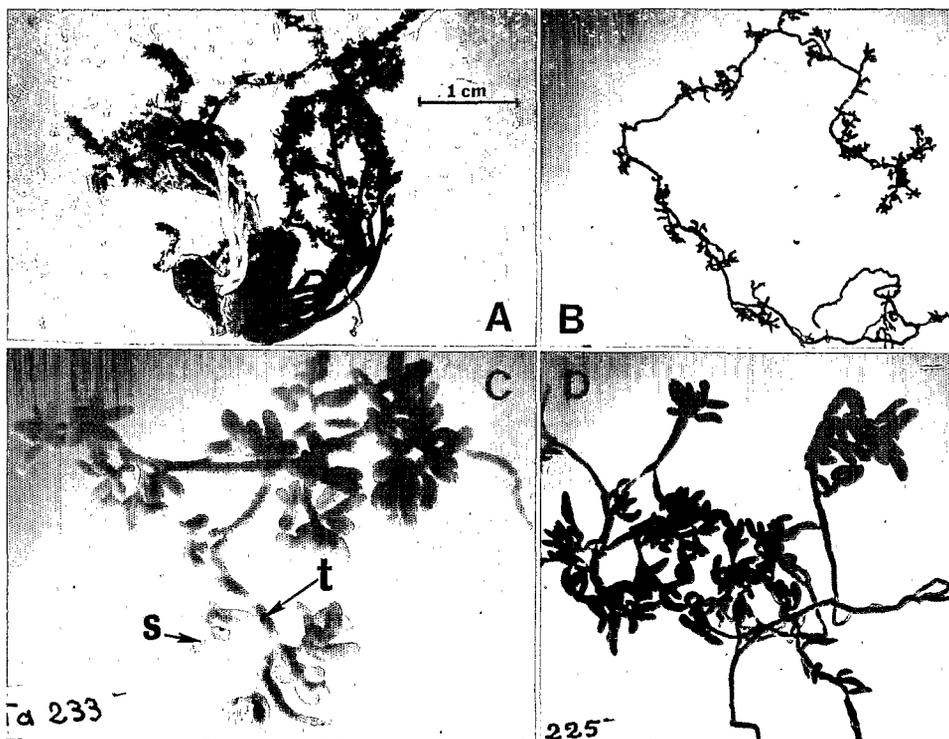


Figure 4. Gonad macroscopic features of *T. ananas*: A, sex undetermined; B, one tubule of male gonad at stage V; C, (lower left) portion of female tubule at stage IV; D, (lower right) portion of a male tubule at stage IV; s, saccule; t, tubule.

Frequency distributions of these characters were established for the 298 harvested individuals of *T. ananas*, 377 *M. nobilis* and 125 *M. fuscogilva*. The relationships between the characters, considered as independent variables, were investigated by regression and correlation analysis.

To study the sexual cycle, the gonads were preserved in buffered formalin at 7% dilution. Since a histological study was considered to be too time consuming, the method used to define the sexual stages of certain fish (Conand, 1975) was employed. This method combines the notation of macroscopic characters of form, color and consistency with the microscopic observation of a fragment of gonad preserved in formalin. Holothurian gonads are composed of tubules, a part of which was examined, opened and spread onto a hollow plate to reveal the sex. In the case of females, the oocyte diameters were measured by means of an ocular micrometer in order to establish their frequency distribution. These approaches permitted five stages of sexual maturity to be defined for each species.

The gonad index (GI) was expressed as the ratio of gonad weight to drained weight. The mean value was calculated for each sample, as well as the confidence limits at the probability level of 0.90. Results were obtained by computer processing.

Mean bimonthly and monthly sea water temperatures were calculated from daily measurements at the Noumea-Anse Vata coastal station (Fig. 2).

The size at first sexual maturity, or size at which an individual may reproduce for the first time, is an important parameter for stock management, but few data are available for holothurians (Choe, 1963; Rutherford, 1973; Harriot, 1980).<sup>1</sup> A method previously applied to fish populations (Conand, 1975), involving construction of a plot of the percentage of mature individuals in each size class, was employed. The percentage of individuals in maturity stages III, IV and V were recorded in classes of drained weight (DW), using both the entire sample and a sample which excluded the resting period, since the number of undetermined individuals was found to be maximal at this time. The size classes at which 0% and 100% of the individuals are mature were determined on the curve. The point on the curve at which 50% of the size class are sexually mature ( $DW_{50}$ ) may be taken as an index of the size at first sexual maturity. This method assumes that the population consists of a single age class, or

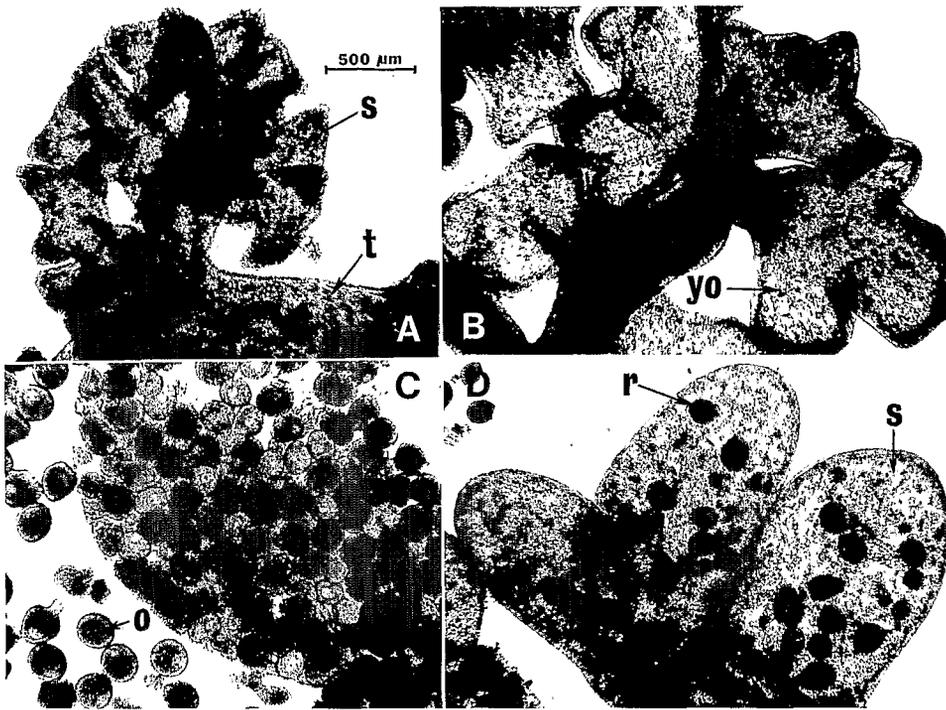


Figure 5. Microscopic aspects of stages of maturity in *T. ananas*: A, sex undetermined; B, female, stage III; C, female, stage IV; D, female, stage V; o, ripe oocyte; r, residual oocyte; s, saccule; t, tubule; yo, young oocyte.

that in a population containing several age classes, older animals at stages III, IV and V will be larger than those reaching sexual maturity for the first time. The total length and gutted weight at first maturity,  $TL_{50}$  and  $GW_{50}$ , were calculated from the regression equations.

## RESULTS

### Distribution and Abundance

*T. ananas* was found at a depth between 8 and 15 m amongst coral patches, on a flagstone covered with a fine layer of sand. The CPUE was approximately 20.

The black teatfish was found in the lagoon, on the inside slope of the Great Reef and close to the islands, and its numbers were often few. Owing to its extremely dispersed distribution in groups of a few individuals, samples were taken at several stations (Fig. 2). At the Amédée Lighthouse, it was sampled on the reef flat, whereas at the other stations, individuals were found on the sand around the base of coral slopes at depths of between 2 and 8 m. The CPUE was rather variable but averaged approximately 12.

The white teatfish is not very abundant in New Caledonia. Adult individuals were mostly found near the passes of the Great Reef, generally above 5 m. In Truk (Caroline Islands) it is distributed in much the same way (Howell and Henry, 1977). In Fiji, on the other hand, it predominates over the black teatfish; Gentle (1979) discovered it along with young forms in superficial seagrass beds of *Syringodium isoetifolium*. At the Uitoé pass in New Caledonia (Fig. 2), individuals were dispersed on the flagstone at depths of between 5 and 30 m. The CPUE,

Table 3. Characteristic features of the maturity stages in *Thelenota ananas*, prickly redfish (n = number of specimens,  $\bar{m}$  = mean, SD = standard deviation)

Maturity Stage Sex	Macroscopic Features	Microscopic Examination		Gonad Weight (g $\times 10^{-3}$ )	Gonad Index
Undetermined Sex	2 tufts of 5 to 12 tubules; tubules short: 1 to 6 cm in length; tubules without branching; budding saccules; color: grey.	sex indistinguishable; germinal cell diameter $\leq 30 \mu\text{m}$ .	$\bar{m}$	3.63	0.029
I Immature			n	51	50
II Resting			SD	2.83	0.014
III Growing male	elongated tubules: 5 to 12 cm in length; increasing size and number of saccules; color: purple.	some spermatozoa can be obtained from a portion of a tubule.  growing oocytes $\leq 150 \mu\text{m}$ , without modal size.	$\bar{m}$	28.09	0.139
female			n	22	22
			SD	19.07	0.072
IV Mature male	30 or more tubules in each tuft; tubule length: 12 to 25 cm; extremely full tubules and swollen saccules rounded in females, more elongated in males; color: purple.	sperm swimming from a section.  oocyte distribution polymodal; mode of ripe oocytes at $200 \mu\text{m}$ filling the lumen, small ones near the gonadal wall.	$\bar{m}$	261.33	1.124
			n	42	40
			SD	159.37	0.525
			female	$\bar{m}$	379.43
n	49	49			
V Post Spawning male	number of tubules decreasing; tubules shortening; tubules and saccules limp in consistency; color: purple to brown.	swimming sperm remaining in some saccules.  a few large oocytes or ruptured follicular membranes remaining; phagocytic cells destroying the residual oocytes.	$\bar{m}$	64.67	0.319
			n	60	60
female			SD	64.64	0.292
			$\bar{m}$	60.88	0.287
			n	35	35
			SD	77.88	0.346

Table 4. Characteristic features of maturity stages in *Microthele nobilis*, black teatfish (n = number of specimens,  $\bar{m}$  = mean, SD = standard deviation)

Maturity Stage Sex	Macroscopic Features		Gonad Anatomy		Gonad Weight (g × 10 <sup>-3</sup> )	Gonad Index	Microscopic Examination
			Tubule L. (mm)	Tubule Ø (mm × 10 <sup>-1</sup> )			
Undetermined Sex I Immature	few transparent tubules with little branching; distal end of tubules round in shape.	$\bar{m}$	8.5	1.5	2.6	0.039	spherical germinal cells with diameter ≤30 μm.
		n	23	23	24	23	
		SD	4.6	0.7	3.5	0.051	
II Resting							
I Growing I male I female	several, more branching, whitish tubules.	$\bar{m}$	64.2	5.4	52.7	0.56	few spermatozoa.
		n	12	12	12	12	
		SD	37.3	1.6	77.2	0.77	
female		$\bar{m}$	28.8	4.8	22.0	0.24	growing oocytes ≤120 μm without modal size.
		n	12	12	12	12	
		SD	14.3	1.9	36.7	0.40	
IV Mature male	maximum volume; off-white numerous tubules with swellings; sperm may be present in gonoduct; swollen translucent numerous tubules; transparent ripe oocytes.	$\bar{m}$	124.2	10.4	423.7	2.89	numerous spermatozoa swimming from a tubule section.
		n	61	59	61	60	
		SD	34.0	2.8	368.7	2.14	
female		$\bar{m}$	105.4	20.2	771.8	5.04	polymodal distribution of oocytes; principal mode of spherical or polygonal oocytes circa 150 μm; secondary mode more or less apparent circa 80 μm.
		n	75	74	75	75	
		SD	34.1	4.83	631.8	3.42	
V Post Spawning male	some tubules as in stage IV but more limp, others shortening and showing atresia; color: brown.	$\bar{m}$	77.9	6.1	122.9	0.95	some spermatozoa remaining; brown or yellowish atretic cells.
		n	102	17	102	98	
		SD	36.3	2.1	119.9	0.82	
female		$\bar{m}$	59.2	9.8	128.4	0.95	a few oocytes of 100–160 μm; brown or yellowish atretic cells; occasionally some empty follicular membranes.
		n	84	80	84	83	
		SD	22.9	5.0	131.5	0.90	

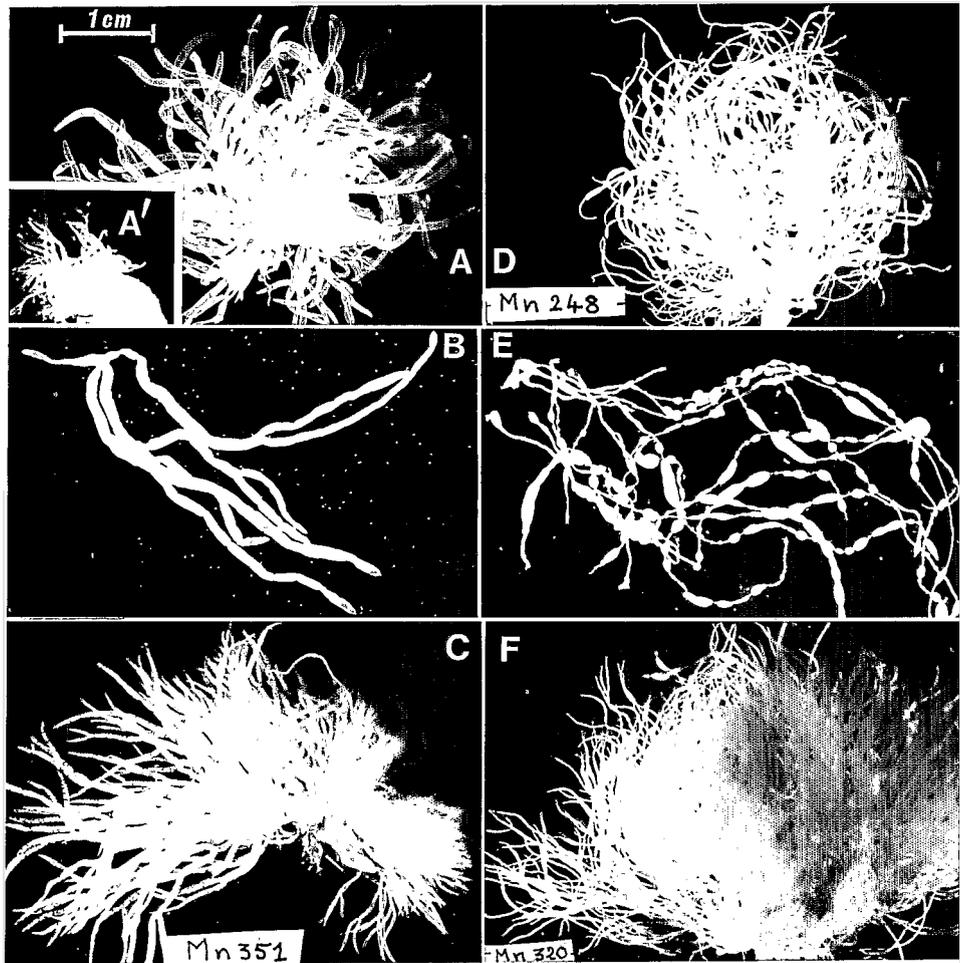


Figure 6. Gonad macroscopic features of *M. nobilis*: A, female, stage III; A', sex undetermined; B, female, stage IV; C, female, stage V; D, male, stage III; E, male, stage IV; F, male, stage V.

which reached 20 at the first samplings, dropped for the last harvests, which probably indicates a depopulation at this station.

#### Size Frequency Distributions and Biometric Relations

The size frequency distributions (Fig. 3 and Table 1) were unimodal and approximately normal for *T. ananas* and *M. fuscogilva*. They reveal the phenomenon of "one size class in a locality" (Bakus, 1973). The majority of specimens encountered were adults, young forms appearing rarely (Fig. 1). Size distributions of *M. nobilis* obtained by grouping the data from the different stations did not appear to be unimodal. A detailed study per station will be conducted at a later date.

The biometric relations established between pairs of characters are presented in Table 2. The variability of biological material has often been noted. This mostly

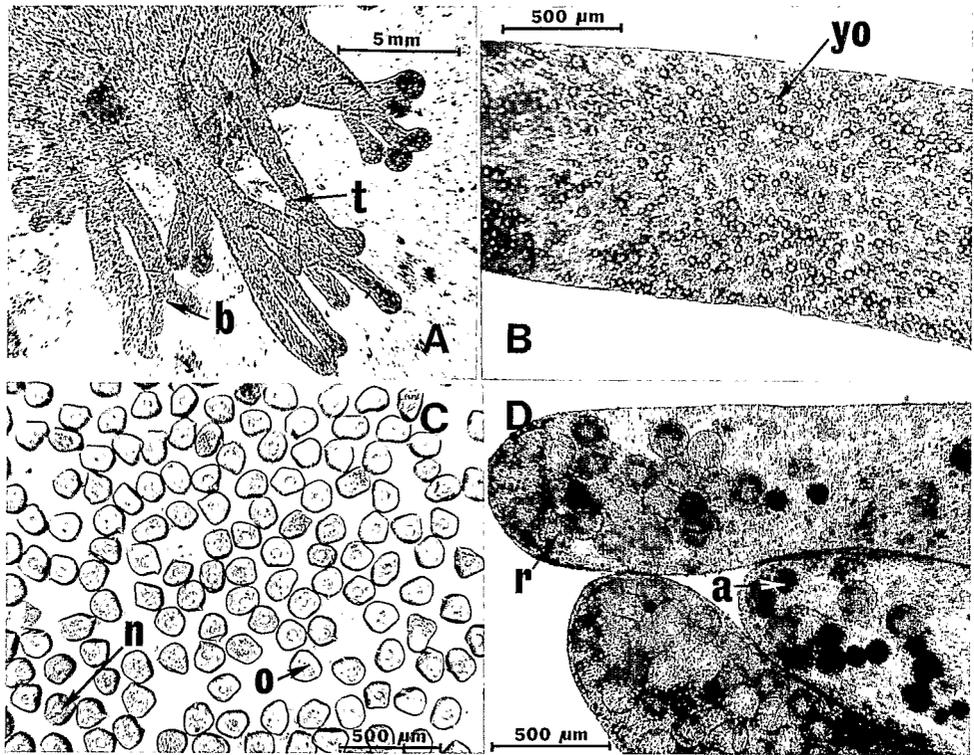


Figure 7. Microscopic aspects of stages of maturity in *M. nobilis*: A, sex undetermined; B, female, stage III; C, female, stage IV; D, female, stage V; a, atretic oocyte; b, tubule branching; n, oocyte nucleus; o, oocyte; r, residual oocyte; t, tubule; yo, young oocyte.

results from the differences between individuals, but in the case of holothurians, there are also numerous inaccuracies when measuring an individual. Length, difficult to determine because of the body wall consistency, also varies with the phase of activity during sampling (feeding or resting). Weight varies according to the amounts of coeliac water and of sediment in the alimentary canal. The values of the correlation coefficients calculated for the relations (1) are rather low. However, only the length can be easily measured in the sea. These variations led Choe (1963), during his study of *Stichopus japonicus*, to choose the gutted weight in calculating the gonad index. The extremely high values of the correlation coefficients for the relations (2) show the distinctness of the relation between the drained and gutted weights and justify the choice of drained weight as characteristic weight for calculation of gonad indices.

#### Gonads, Sexual Stages and Sex-ratios

The gonads of *T. ananas* are composed of two tufts of tubules on which expansions or saccules develop. The epithelium of the gonads contains a red pigment which often makes it difficult to determine the sex, and it also contains several spicules similar to those of the body wall.

The characteristics of the five stages are presented in Table 3 and illustrated by Figures 4 and 5. Stages I and II comprise individuals of undetermined sex,

Table 5. Characteristic features of the maturity stages in *Microthele fuscogilva*, white teatfish (n = number of specimens,  $\bar{m}$  = mean, SD = standard deviation)

Maturity Stage Sex		Gonad Tubules		Gonad Weight (g $\times 10^{-3}$ )	Gonad Index
		L (mm)	$\Phi$ (mm $\times 10^{-1}$ )		
Undetermined	$\bar{m}$	9.3	1.7	1.87	0.017
I Immature	n	16	16	16	14
	SD	8.1	1.2	1.54	0.010
II Resting					
III Growing male	$\bar{m}$	45.8	4.0	28.33	0.20
	n	9	9	9	9
	SD	22.2	0.5	44.39	0.31
female	$\bar{m}$	33.5	8.1	26.50	0.18
	n	10	10	10	10
	SD	9.4	2.2	23.17	0.14
IV Mature male	$\bar{m}$	88.0	9.0	137.67	0.83
	n	27	27	27	27
	SD	25.6	3.4	150.06	0.80
female	$\bar{m}$	78.6	15.7	359.57	2.18
	n	21	21	21	20
	SD	25.2	6.6	306.48	1.74
V Post Spawning male	$\bar{m}$	50.6	4.1	26.14	0.17
	n	21	21	21	21
	SD	29.2	2.2	23.69	0.16
female	$\bar{m}$	47.6	12.2	67.47	0.45
	n	19	19	19	19
	SD	21.8	16.9	74.48	0.55

stage I corresponding to immature individuals and stage II to the resting ones. The characteristics of the gonads are identical and only the determination of the size at first sexual maturity makes it possible to distinguish individuals of these two stages. Stage III corresponds to the early maturation; the sexes can therefore be distinguished by a microscopic examination. Stage IV becomes evident by the increased volume of the gonads and includes mature and spawning individuals. The ripe oocytes measured approximately 200  $\mu\text{m}$ . The average gonad index was 1.1% for males and 1.6% for females. Determining the post-spawning stage V is more difficult as the characteristics of the gonad weight and the length of the tubules can be compared to those of stage III. However, the gonads are deflated and more limp and residual ripe oocytes or spermatozoa may be observed as well as signs of atresia and resorption of germinal cells by phagocytic cells.

In *M. nobilis* the sexes are separated: the ovaries and the testes are composed of one tuft of tubules, each of which generally has 2–3 branches (Fig. 6). Their length and diameter vary during the reproductive cycle and are useful in determining the maturity stage (Table 4). Gonads present a sexual dimorphism: in females the tubules are shorter and wider and at stage IV the ovary weight and gonad index value are higher (5.0%) than in males (2.9%). Distinguishing stages III of maturation and V of post-spawning is generally easier than for *T. ananas* since the pigmentation of the gonads, which varies according to the species, is lighter. The ripe oocytes of *M. nobilis* measured from 140 to 160  $\mu\text{m}$  (Fig. 7).

The gonads of *M. fuscogilva* have the same structure as those of *M. nobilis*

(Figs. 6 and 7). The sexes are separated. Table 5 gives the mean values of the length and diameter of tubules and the gonad weight and gonad index calculated for the different stages in *M. fuscogilva*. Its gonads present the same sexual dimorphism as *M. nobilis*. At stage IV the mean gonad index was 2.2% for females and 0.8% for males. The ripe oocytes measured approximately 170  $\mu\text{m}$ .

The monthly percentages of male, female and undetermined individuals were calculated (Fig. 8). No hermaphrodite individual nor any sign of asexual reproduction by transverse fission was observed. The undetermined individuals represented 17% of the entire sample of *T. ananas* but their percentage was higher in July and August 1979. The sex-ratio was 1.08:1.0 (males : females). The 24 undetermined individuals of *M. nobilis* represented only 6.5% of the total but their percentage was higher in December 1978 and 1979 when they exceeded 20%. The sex ratio was 1.02:1.0. In *M. fuscogilva*, the undetermined individuals represented 13% of the total but their percentage was slightly higher in April. The sex ratio was 1.14:1.0.

### Reproductive Cycles

For *T. ananas* monthly percentages of individuals at stages III, IV, and V showed a similar pattern in both males and females (Fig. 9). During October, November, and December 1978, maturing individuals (stage III) were preponderant and the percentage of mature individuals increased. During January, February, and March 1979 there were no more individuals at stage III and the majority was composed of mature individuals; however the percentage of post-spawning (stage V) individuals increased. From April to August 1979 stage V became predominant, with an increase in the percentage of maturing individuals paralleling the decline of stage V. There was a similar cycle from October 1979. The spawning period was therefore spread out from December to April with a peak during the first 3 months. The high percentage of individuals of undetermined sex observed in July and August (Fig. 8) may therefore partly correspond to adults in the resting period.

The average monthly gonad index (Fig. 10) showed seasonal variations. There was a period of increasing values from October to January, a decline from February to June followed by low values from July to October. This substantiates those observations obtained from the study of the gonads. The variances of these values were quite high during the reproduction period, indicating a high individual variability of the state of maturity. The variances declined sharply from May to October when the entire population under study arrived at the spent or resting periods.

The annual variation of the sea surface temperature at the Anse Vata coastal station (Fig. 10) showed an alternating warm season and cold season with transitional season in between. By associating the sexual cycle with the temperature, maturation was observed to begin during water heating in September. Spawning occurred during the warm season and post-spawning, followed by resting, predominates during the cooling period from April to September.

*T. ananas* therefore presents an annual reproductive cycle marked by one single spawning period during the warm season.

During the year, males and females of *M. nobilis* developed in the same way (Fig. 9). Maturing individuals were only found from January to May and were at a maximum in March. The pre-spawning and spawning periods followed until November, with a maximum in May–June–July. Post-spawning individuals were found virtually throughout the year but predominated from November to Feb-

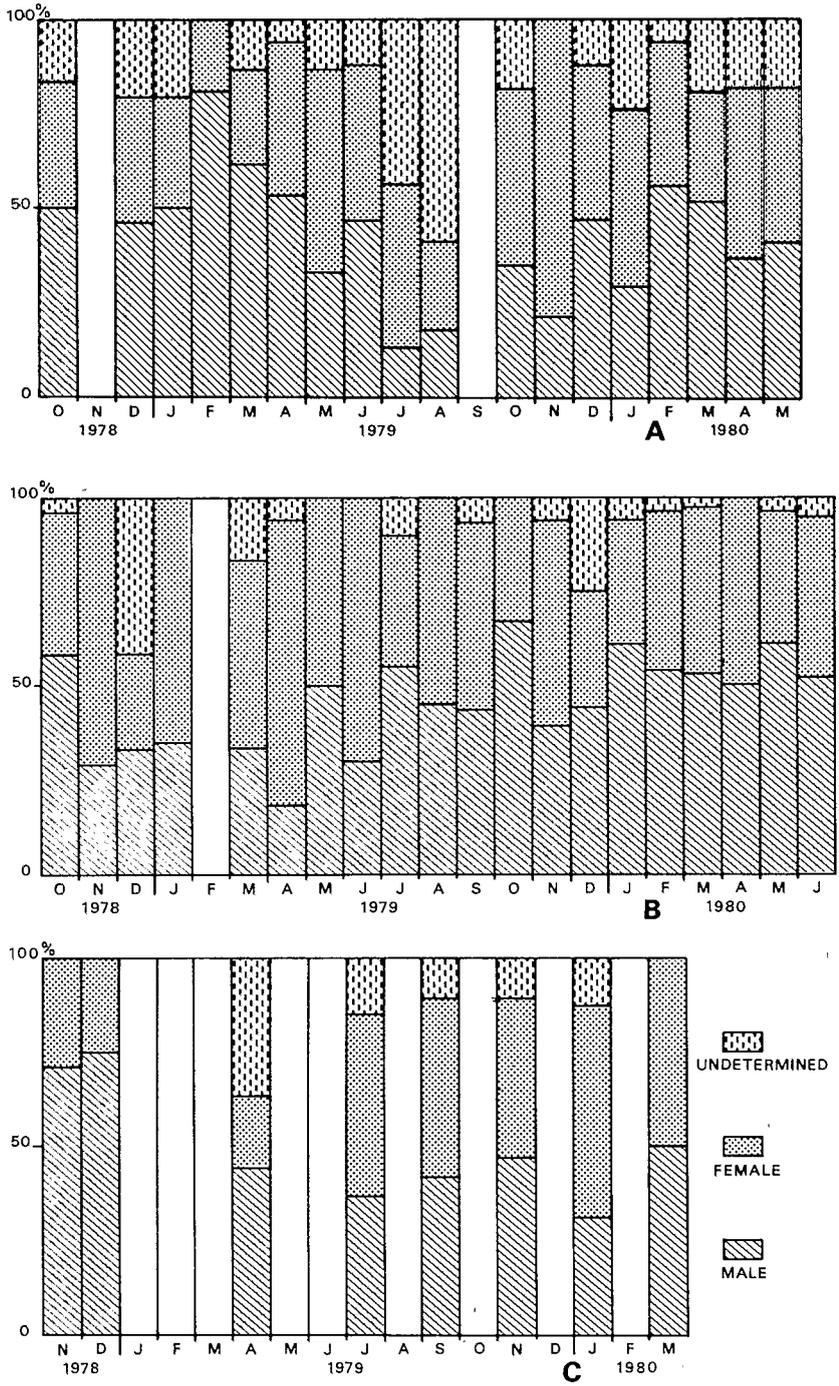


Figure 8. Monthly percentages of males, females and undetermined individuals. A, *T. ananas*; B, *M. nobilis*; C, *M. fuscogilva*.

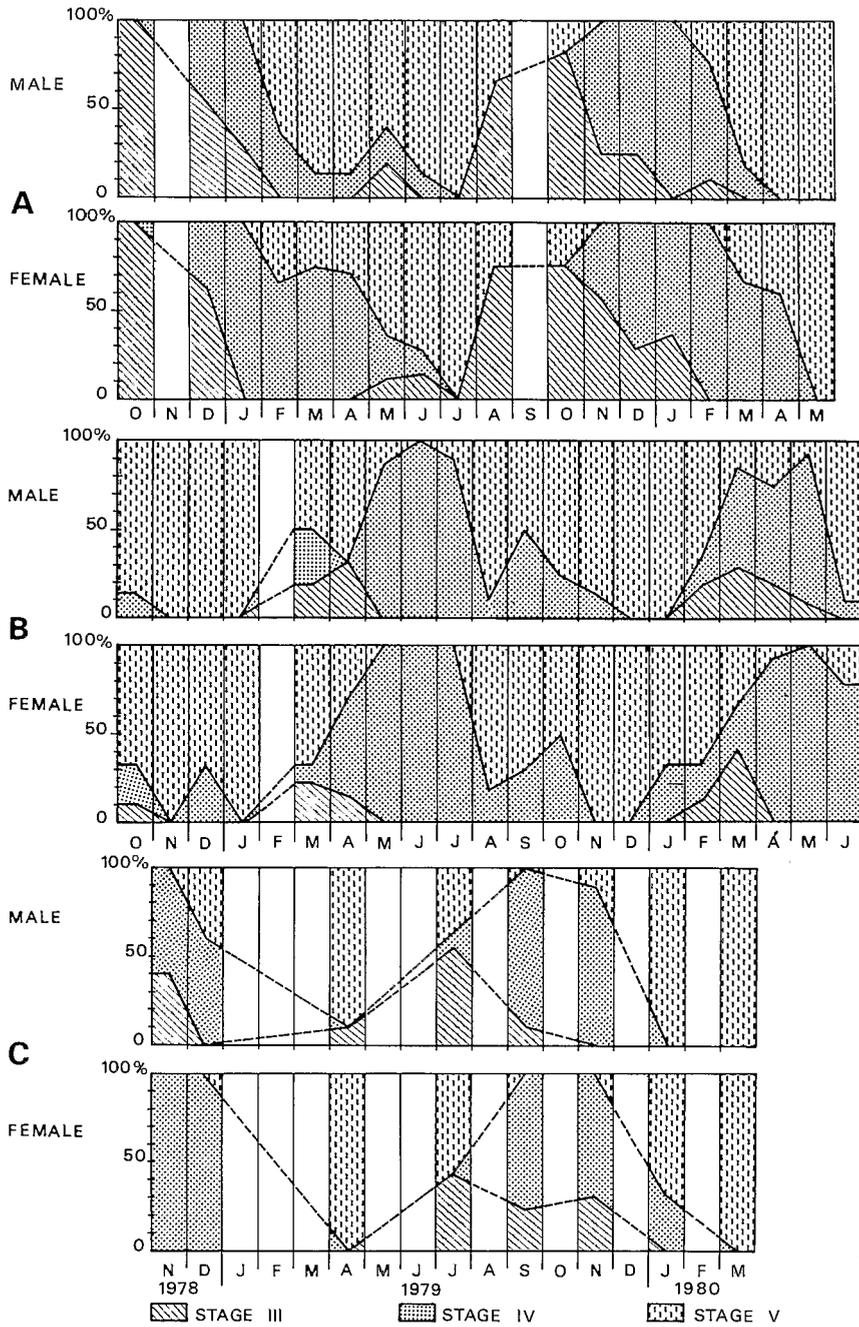


Figure 9. Monthly percentages of maturity stages. A, *T. ananas*; B, *M. nobilis*; C, *M. fuscogilva*.

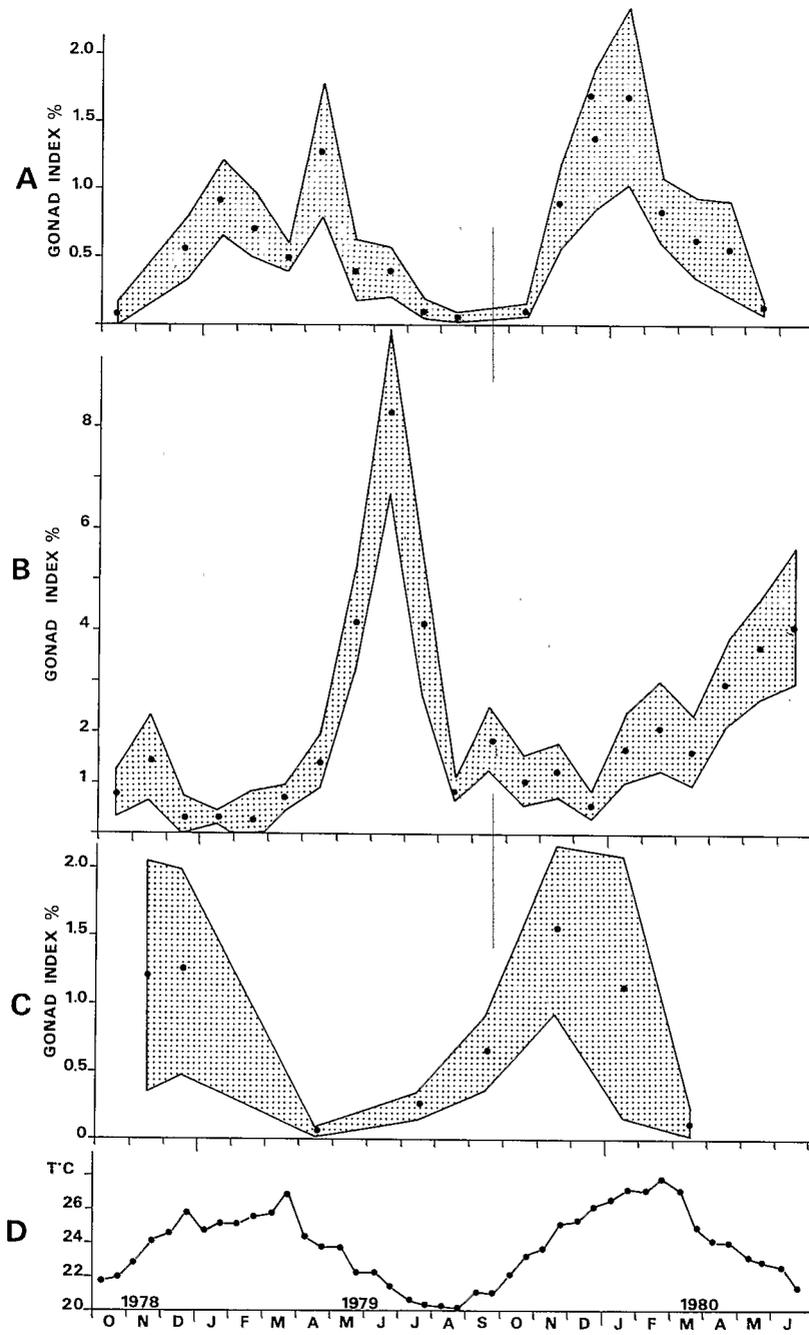


Figure 10. Seasonal changes of gonad index and surface water temperature. The points are the mean gonad indices, the bands are the 0.90 confidence limits. A, *T. ananas*; B, *M. nobilis*; C, *M. fuscogilva*.

ruary. The high percentage of immature individuals in December corresponded to the maximum number of post-spawning individuals. Generally, results from two consecutive years tally.

The average monthly gonad index (Fig. 10) showed a very high peak in May–June–July 1978 and a slightly lower peak in May–June 1979. The sudden decline of the gonad index in July 1978 corresponded to the beginning of the spawning period which probably lasted until November. In fact, sperm was observed in the male gonoduct throughout this period. In this species the resting phase took place during the warm season.

*Microthele nobilis* therefore presents an annual reproductive cycle marked by a long spawning period during the cold season and perhaps while the waters are warming; maturation and resting periods are brief. Observations by Mortensen (1938) made during the July–September period in the Red Sea (warm season) indicated that reproduction took place during this time, a fact which differs from our results. It is questionable whether this is the same species, since the Red Sea species has a body color and an anal papillae morphology quite different from the New Caledonian species (Cherbonnier, 1955).

The cycle of different maturity stages (Fig. 9) is not defined in as much detail for *M. fuscogilva* as for previous species because of the reduced sample size. Gonad growth lasted from April to November and the maximum percentage of individuals at stage III is in July. Pre-spawning and spawning stages showed a similar pattern in both sexes. The post-spawning stage lasted from September to January and the highest percentage of undetermined individuals was recorded during this period.

The mean gonad index (Fig. 10) showed two similar peaks in November–December 1978 and in November 1979 followed by a rapid decline in April 1979 and March 1980. A small confidence interval corresponded to the low values of the gonad index. The gonad index increased slowly from May to September. As in the two other species studied, the synchronism between all individuals only occurred during the resting stage which, in the case of *M. fuscogilva*, was when the waters were becoming cold from March to June. Maturation occurred during the cold season and while the waters were warming again. Most individuals spawned at the beginning of the warm season in January and February. This cycle is therefore very different from that of the black teatfish.

#### Size at First Sexual Maturity

For *T. ananas* the percentages of mature individuals in drained weight classes increase between 350 and 1,850 g; the weight at first maturity,  $DW_{50}$  equals 1,200 g when the entire sample series is used and 1,150 g when the resting period is excluded (Fig. 11). From this may be calculated  $TL_{50} = 300$  mm and  $GW_{50} = 957$  g.

Similarly, for *M. nobilis* the first maturity is reached between 250 and 850 g DW;  $DW_{50} = 580$  g,  $TL_{50} = 227$  mm and  $GW_{50} = 474$  g (Fig. 11). Individuals showing a great deal of cream color on the black body were mostly immature (10 from 18 specimens observed) and all individuals weighing under 500 g had these pigments. It is therefore noted that sexual maturity appears at the same time as the pigmentation of the young form disappears.

For *M. fuscogilva* the first maturity is reached between 700 and 1,500 g DW;  $DW_{50} = 900$  g,  $TL_{50} = 324$  mm and  $GW_{50} = 779$  g (Fig. 11).

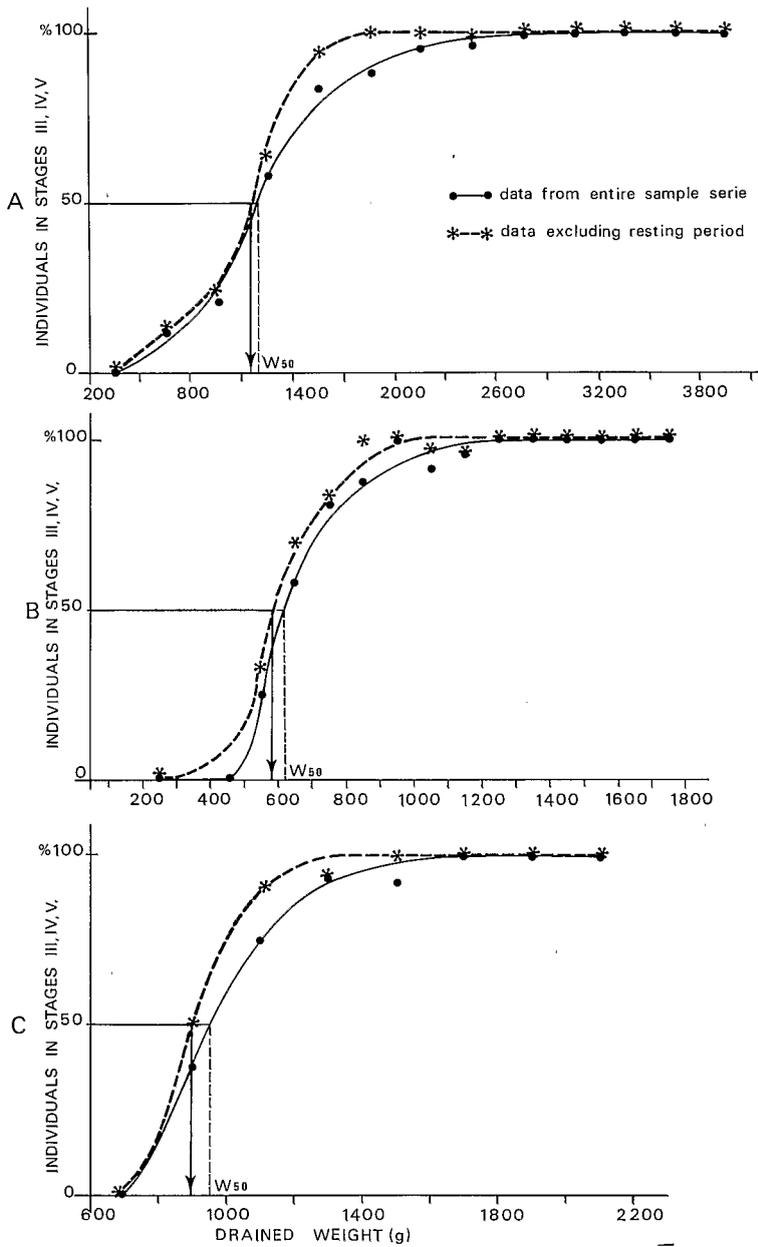


Figure 11. Weight at first sexual maturity. A, *T. ananas*; B, *M. nobilis*; C, *M. fuscogilva*.

## DISCUSSION

### Reproductive Cycle

This first study of the sexual cycle of commercially viable holothurians in New Caledonia proves the complexity of the phenomena. A coherent outline can only

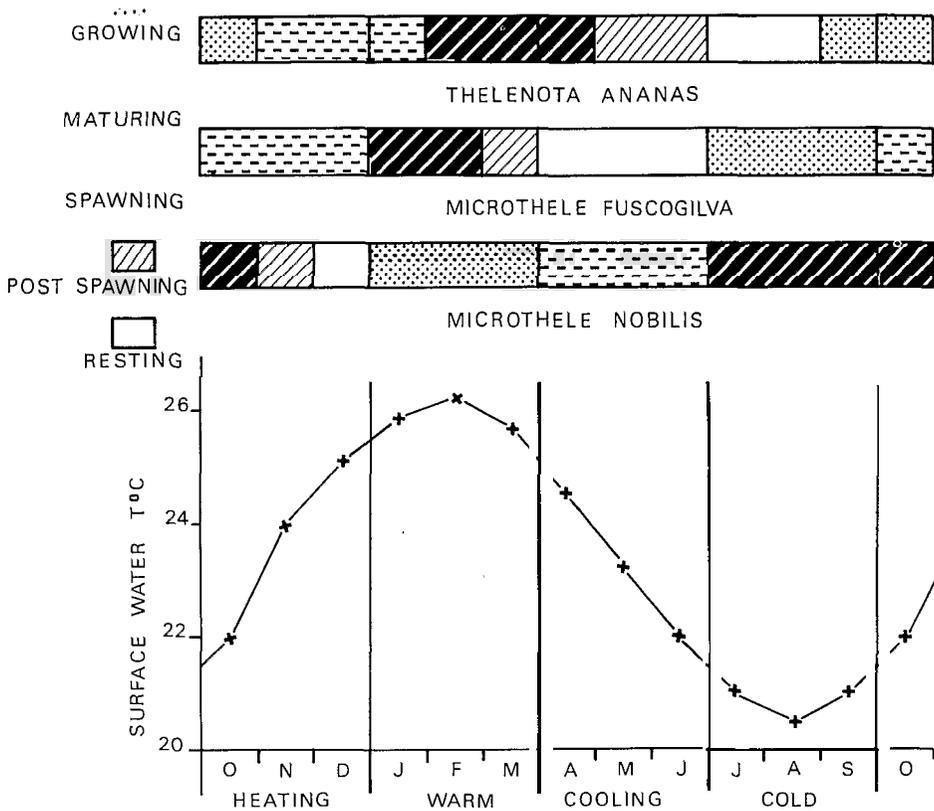


Figure 12. Phases of the reproductive cycle of some holothurian species related to the sea water temperature.

be obtained through the combined use of several techniques. The reproductive cycle of each species can only be elucidated by simultaneous observation of variations of the gonad index and its confidence limits, the cycle of different maturity stages and the percentage of undetermined individuals (Fig. 12). The peak period of stage IV is dissociated into maturing or pre-spawning (maximum peak of gonad index) and spawning (beginning of the decline of the gonad index). The peak period of stage V is divided into post-spawning or spent (during the decline of the gonad-index) and resting (corresponding to the maximum percentage of undetermined individuals). *TheleNOTA ananas* and *M. fuscogilva* reproduce in the warm season whereas *Microthele nobilis* spawns during the cold season and at the beginning of the water warming period. The three species therefore exhibit an annual reproductive cycle although they present wide variations in seasonality.

Harriot (1980)<sup>1</sup> in her study of the holothurian fauna of Heron Reef found different reproductive patterns in three congeneric species: *Holothuria atra* spawns biannually in summer and winter, *H. impatiens* annually in spring, and *H. edulis* without annual cycle apart from a summer resting period. It is interesting that the two congeneric species of the present study show differences in season-

ality and no overlap in their spawning periods (Fig. 12). The spawning of *T. ananas* and *M. fuscogilva* during the warm water season conforms with the most common reproductive pattern.

### Fecundity

Comparing the mean values of ovary weights and ripe oocyte diameters (Tables 3, 4, 5) makes it possible to classify species according to their total individual fecundity or the number of ripe oocytes found in the ovary. *M. nobilis* produces small oocytes in large numbers since the ovary weight is very high, whereas *T. ananas* and *M. fuscogilva* have lighter ovaries and more voluminous oocytes. A coefficient of fecundity may be estimated by dividing the mean weight of the ovary at stage IV by the cube of the oocyte diameter; it equals 4,750 for *T. ananas*, 22,800 for *M. nobilis* and 7,350 for *M. fuscogilva*. The inverse relationship between ovum size and number, hypothesized by Stearns (1976) and valid for the reef holothurians studied by Harriot (1980),<sup>1</sup> is also confirmed for these species.

The gonad index values at stage IV make it possible to compare relative fecundity or fecundity related to body weight. In the present study, *M. nobilis* revealed the highest values, 3.5 times greater than for *M. fuscogilva* and 6.0 times greater than for *T. ananas*.

### Size at First Sexual Maturity

The size at first maturity has been observed for some holothurians: the smallest mature *Stichopus japonicus* had a body wall weight of 39 g but in general they weighed 58–60 g (Choe, 1963); *Cucumaria pseudocurata* probably becomes reproductive below 23 mg (Rutherford, 1973); mature gonads were rarely found in *Holothuria atra* weighing less than 100 g (Harriot, 1980).<sup>1</sup>

The method used in the present study to determine the size at first maturity gives precise results allowing comparisons between the species. In *M. nobilis* first maturity is reached at a lesser size and weight than in *M. fuscogilva* and *T. ananas*. Related values between the first maturity weight and the mean weight ( $DW_{50}/\bar{DW}$ ) were 0.46 for *M. nobilis*, 0.58 for *T. ananas* and 0.61 for *M. fuscogilva*. Growth studies will be necessary to determine the age at first maturity. In order to deal with this problem, a sufficient number of young individuals must be found to study their development or tag them.

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ADDRESS: Centre ORSTOM, section océanographie, B.P. A5, Nouméa, Nouvelle-Calédonie.