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Spécialité : Biodiversité, Écologie et Évolution (BEE)

## **SEACUSEY PROJECT**

# **Sea cucumber fishery in Seychelles: size at sexual maturity of *Holothuria (microthele) pentard***



Par CAHUZAC Salomé  
Mai 2018

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Responsable et co-responsable de stage : Mr Govinden Rodney et Mme Conand Chantal

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Size at sexual maturity of the flowerfish  
*Holothuria (microthele) pentard* in Seychelles

..

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## Charte anti-plagiat

Je soussignée, CAHUZAC Salomé, étudiante en première année de master SET spécialité BEE à Aix-Marseille Université,

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Fait à Riantec, le 25/05/2018.

Signature



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Je tiens à exprimer toute ma reconnaissance envers les personnes qui m'ont soutenue durant la réalisation de ce travail.

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# Introduction

Holothurians, also known as sea cucumbers, are marine invertebrates from the family of Echinodermata, which are distributed throughout tropical and temperate waters. They have been fished in the Seychelles (southwest Indian Ocean) for more than one hundred years. Holothurians are mostly collected by divers using scuba gear at 15 m to 45 m depth. They are further processed and dried for the Asian export market as “bêche-de-mer”. Holothurian resources have globally been overexploited due to the rise in the Chinese market since the 1990s. In the Seychelles, sea cucumber harvesting was run as an open access fishery up to the end of the 20<sup>th</sup> century, without any control or any regulations by the fishery authority due to the low catch. However, in 1999, as a response to the local depletion of some species, the Seychelles Fishing Authority (SFA) established a management plan for a sustainable fishery (Conand & Muthiga 2007). This plan was finalized in 2005. The teatfish *Holothuria* group (*H. fuscogilva*, *H. nobilis*, *H. (Microthele)* sp. “pentard”) is a group of sea cucumber species that are heavily targeted in the Seychelles as they are among the most expensive species (Purcell and al 2012, 2017; Diassanayake and Gunnar, 2010). With more than 200 000 individual sea cucumbers harvested per year, the flower teatfish *Holothuria* sp. (called locally “Pentard”) is the main commercial species in the Seychelles, while *Thelenota ananas* (average of 60 000 pieces per year) and *H. fuscogilva* (average of 50 000 pieces per year) ranging next (Aumeerudy & Conand, 2008; SFA, pers. com.). The commercial value of the Pentard has progressively led to the overexploitation of the stock in the Seychelles (Conand & Muthiga, 2007; Purcell and al 2017).

The Pentard has been observed in Seychelles, Comoros, Tanzania, Madagascar, Sri Lanka and Maldives (Purcell and al 2012). However, to date, that species has not yet been formally described although work is going (Paulay G., pers. com.). Information on the ecology of Pentard is poorly known, which makes it difficult to model local population dynamics and to design suitable management or conservation measures. For instance the introduction of a minimum catch size could be beneficial both economically and ecologically.

The present work was performed as part of the SEACUSEY project, whose purpose is to ensure the sustainability of the economic sector related to sea cucumber resources in the Seychelles through adaptive co-management. This study aims to better understand the reproduction behavior of Pentard and, specifically, to determine the size at first sexual maturity in order to improve the fishery management. It has been realized with the helpful collaboration of the fishers.

# Materials and methods

## *Survey area and sampling*

The survey was carried out at two sites, namely Frégate island on the Mahé Plateau (02/02/2108) and close to Marie-Louise island on the Amirantes Plateau (07/03/2018) on board “L’Amitié”, SFA’s research vessel (Fig 1).

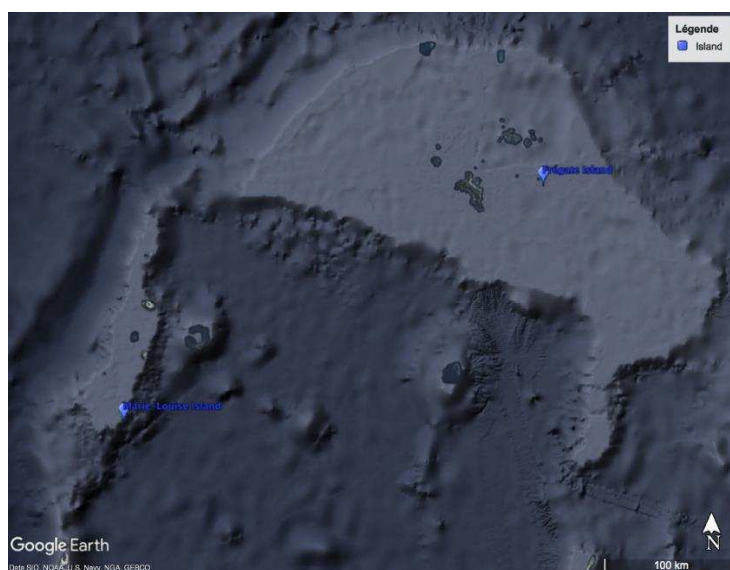


Figure 1: Sampling sites

Due to human and material limitation, a collaboration with sea cucumber fishers was successfully launched to collect the samples. The scientific team went on fishing boat in order to do the sampling. Voluntary fishers collected pentard specimens randomly along their way while scuba diving at different fishery sites around Frégate and Marie-Louise islands at around 25-30m depth. They dived in pair for approximately 25 minutes, between three and five times per day. They manually harvested the holothurians and they put them in a net. All the specimens of pentard collected were weighted and measured on the fishing boat directly after being harvested. The total body length (TL) was measured from mouth to anus with a metric tape to the nearest 0,5 cm, and weighted to the nearest 1g using an electronic balance without anti rolling system.

The fishers then incised them dorsally (Fig 2). The entire gonad was removed, weighted to the nearest 1g and further stored in individual tubes of 50mL filled with 10% formalin.



After the removal of internal organs and excess of coelom fluid, the gutted body weight was determined to the nearest 1g.



Figure 2: Fisher opening dorsally an *Holothuria Microthele pentard* freshly harvested on the fishing boat

The sexual stage was determined using the procedure defined by Conand (1981) as follows.

The gonad index (GI) was calculated using the gonad weight by total body weight ratio:  $GI = W_{\text{gonad}} \times 100 / W_{\text{tot}}$ . The GI was expected to be maximal when gonads were mature.

Five maturity stages were established according to morphological parameters of the gonad's tubules: immature (I), resting (II), growing (III), mature (IV) and post-spawning (V). Holothurians were defined as mature between stage III and V. The sexes are separated in this species.

A macroscopic description of the gonads was conducted at the Seychelles Fishing Authority laboratory (Appendix 1). Each gonad was examined and ranged accordingly. The tubule length and width, presence of gametes, and size of oocytes of the females were recorded. First, each gonad sample was placed in a Petri dish. The approximate longest tubule was measured with a measuring tape to the nearest 0.1mm. Second, the tubules were further observed on digital pictures using a trinocular microscope (x1.8 zoom). Between three and five tubules per gonad were observed to define the average width using the caliper of metric system of the Motic image plus 2.0 software. Third, tubules were cut using a scalpel and the fluid found inside was put on a concave blade to determine the sex. For females, the diameter of approximately ten oocytes was measured, using the same software.

Finally, the size at first sexual maturity (L50) was defined as the length at which the gonads of 50% of individuals are matures (Conand 1981; Conand & Muthiga 2014; Navarro and al. 2012). It is the size at which an individual may reproduce for the first time. This data is important to

improve the management of the stock and help to establish a sustainable fishery plan (Conand 1981, Purcell and al. 2018). This determination of the size at sexual maturity of *M. pentard* could be a first reference to better manage the stock of the species in the future.

### *Data analysis*

All data were analyzed in R Studio Version 1.1.442.

The Kruskal Wallis non-parametric test was used to assess the differences between the weight and the gonad morphological parameters.

An increasing cumulative abundance curve of mature individuals (from stage III to V) was built over the total length range. The point on the curve at which 50% of a size class is mature was considered as an index of the size at sexual maturity (Conand 1981, 1989).

As a comparison, a logistic regression of the maturity size was also used to determine the size at sexual maturity through mathematic modeling.

## **Results**

Pentard is the most abundant sea cucumber fished around Seychelles. Before 2001, pentard was recorded in the same fishery group than the prickly redfish (*Thelenota ananas*) because it was a small part of the fishery. They have been separated when their importance in the catch increased. Then pentard has become the main species targeted by the fishers. Some of them have reduced their effort on other species to concentrate on this highly demanded species (Aumeeruddy & Conand 2008).

In this study, a total of 92 *H. pentard* were sampled (31 in les Amirantes and 61 in Frégate Island).

A total of 54 gonads (88,5% of the sea cucumbers collected) from this specimen were harvested on Mahé Plateau and 9 gonads (29%) in les Amirantes Plateau. No gonad was observed for other specimens during sampling, suggesting that their weight was at least as low as the smaller gonad sampled in those areas, respectively (see below).

In the Amirantes plateau, the length and whole individual weight ranged from 200 to 315 mm (mean 263 mm, SD 26 mm) and from 563 to 2153 g (mean 1068 g, SD 38 g), respectively. The Frégate's population reached slightly higher size values than in the Amirantes site. The length and whole individual weight of *Pentard* at Frégate site ranged from 294 to 417 mm (mean 345 mm, SD 25 mm) and from 1032 to 2098 g with a mean of 1644 (s.d. 240 g) , respectively

(Figure 3). However this difference was not significant (Kruskal Wallis test,  $p(\text{weight})=0,36$ ,  $p(\text{length})=0.06$ ).

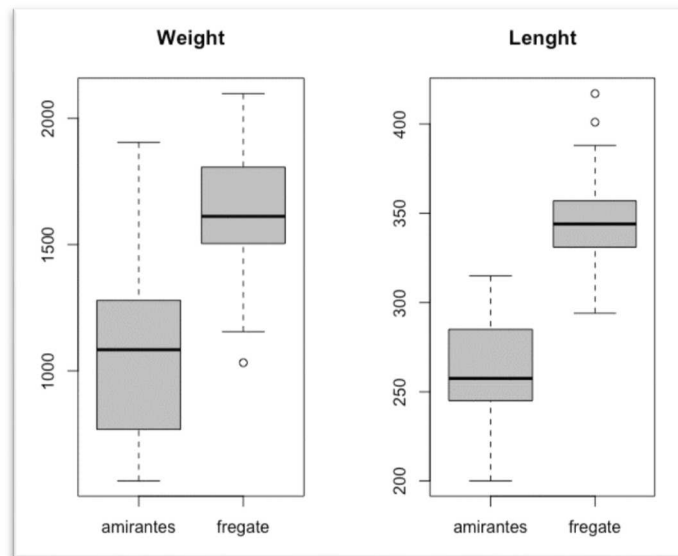


Figure 3: Difference total weight (g) and length (mm) between the two sampling sites (Amirantes and Frégate)

### *Gonad morphology*

Pentard gonads consisted of numerous tubules from a gonad basis, attached on the left of the mouth on the anterior part of the body wall, typical of holothurians (Demeuldre & Eeckhaut 2012). With an external examination, it is not possible to distinguish males and females, there is no observable sexual dimorphism on the entire animal (Ghobadyan and al. 2012). The gonad weight of male and female individual specimens was not significantly different (Kruskal Wallis test,  $p \text{ value} = 0.35$ ).

The characteristics of the five maturity stages were described (table 2, figure 4).

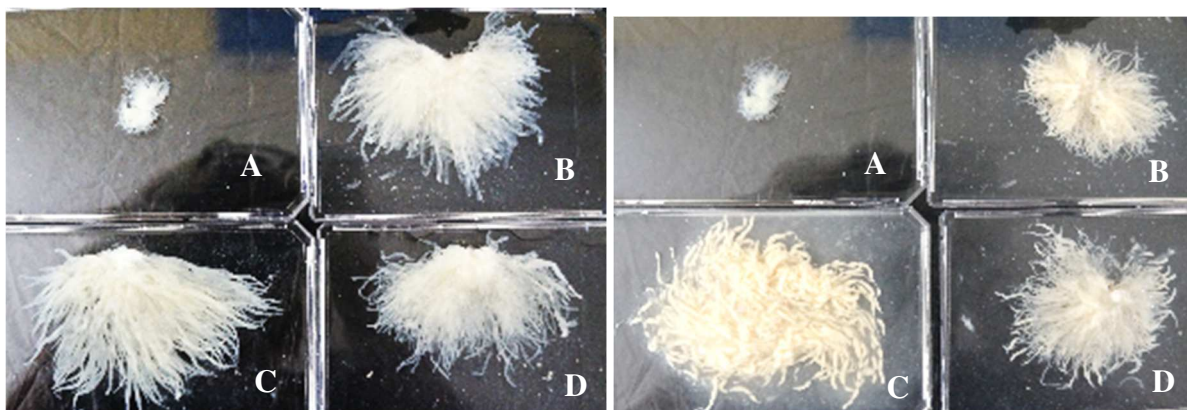


Figure 4 : Left: Female gonad of pentard, Right: Male gonad of pentard

A: immature (stages I-II), B: stage III, C: stage IV, D: stage V

As already reported in Conand (1981), the variability of parameters was important. This resulted from the differences between individuals but also from the several inaccuracies during the measurement at rough sea conditions. The length was variable, it depended on the stress state of the individual sea cucumber. The total weight was influenced by the water in the coelom cavity and the sediment in the intestine. Furthermore, the total weight had been prejudicial by the balance which was not an anti-roll balance. These problems might have been solved by soaking the animal in a solution of chloride magnesium to relax them and measuring them on land, but it was not possible in this situation, animals were intended to the processing factory in order to sell them on the Asiatic market.

Furthermore, the average value of size of tubules, gonad index, gonad weight and oocyte size followed the same profile during the maturity stage: they increased until the stage IV mature and decreased during the stage V (Conand 1981; Hamel & Mercier 1996; Muthiga & Kawaka 2009; Kohler and al 2010; Navarro and al. 2012).

- Stage I and II: Because we did not know the size at first sexual maturity and only used macroscopic observation, it was not possible to distinguish stage I (immature) from stage II (resting). At this step, the sex could not be determined.
- Stage III: the maturation had started, with growing gonads, and exposed and branching tubules. The female gametes oocytes were small but visible, while only unclear liquid was observed within male gonads.
- Stage IV: the gonads were fully grown and mature. Female tubules were filled with oocytes and male tubules had several bulges filled with sperm.
- Stage V: although gonads were broadly similar to those of the third stage, they displayed smaller gonad size and dropped tubules typical of post spawning stage. Some atresian oocytes were also present.

In order to know the differences between males and females, a had been used, the results are presented in the table 1. All the characteristics measured showed were significantly differences between males and females and between mature and immature individuals (Table 1, Figure 5).

Table 1: Statistical comparison (Kruskal Wallis test, p-values) of tubule length, tubule width, gonad index and gonad weight between male and female specimens and between mature and immature individuals.

	<i>Tubule length</i>	<i>Tubule width</i>	<i>Gonad index</i>	<i>Gonad weight</i>
<i>P value of kruskal wallis test between male and female</i>	$2 \cdot 10^{-7}$	$5 \cdot 10^{-4}$	$10^{-5}$	$6 \cdot 10^{-5}$
<i>P value of kruskal wallis test between mature and immature</i>	$3 \cdot 10^{-5}$	$9 \cdot 10^{-6}$	$8 \cdot 10^{-6}$	$9 \cdot 10^{-6}$

Table 2: Macroscopic characteristics of gonads at each maturity stages of *Holothuria Microthele* pentard. Values are presented as mean +/- SD, n= number of sea cucumber individual.

	Macroscopic morphology of tubules	Tubule characteristics					
		Sample (n)	Lenght (mm) ±SD	Width (mm)±SD	Gonad index (%) ±SD	Oocytes size (mm)±SD	Gonad weight (g) ±SD
Stage 1 : Immature Stage 2 : Resting	Tubules small, short, thin and low branching Tubules with uniform width Tubules translucent No visible gametes	9	18.63±9.38	0.24±0.06	0.11±0.03	-	1.5±0.5
Stage 3 : Growing	<b>Males :</b> □ Tubules elongated Emergence of bulges Visible branching Whitish <u>Sperm flow during opening</u>	8	55.37±18.69	0.76±0.23	1.20±0.9	-	20.9±17.3
	<b>Females :</b> □ Tubules elongated Same width along the whole tubules Visible branching Tubules translucent Different size of oocytes appears during opening	7	46.97±13.09	1.14±0.71	0.9±0.2	0.12±0.01	13.6±2.4
Stage 4 : Mature	<b>Males :</b> □ Maximal lenght and width Bulged on the whole tubules Maximal branching Whitish to beige <u>Sperm flow during opening</u>	4	64.16±19.55	1.44±0.53	5.2±2.3	-	81.2±66.8
	<b>Females :</b> □ Maximal lenght and width Tubules larges and swollen Maximal branching Tubules translucent	7	53.7±13.46	1.63±0.30	2.4±1.5	0.16±0.02	41±29
stage 5 : Post spawning	<b>Males :</b> □ Tubules long and branching Some tubules with same aspect than stage 4 Tubules have white spot Yellowish	12	50.36±19.82	0.65±0.29	0.9±0.4	-	14.8±5.4
	<b>Females :</b> □ Tubules long and branching Tubules flask, filled with liquid Some tubules with same aspect than stage 4 Tubules translucent Presence of relict oocytes	10	50.33±11.44	1.17±0.43	0.9±0.5	0,16±0.01	14.2±7.8

As indicated in table 1 and 2 and figure 5, those variables are also significantly different between mature and immature individuals. In this case, the sample size was larger, the results were more strengthfull.

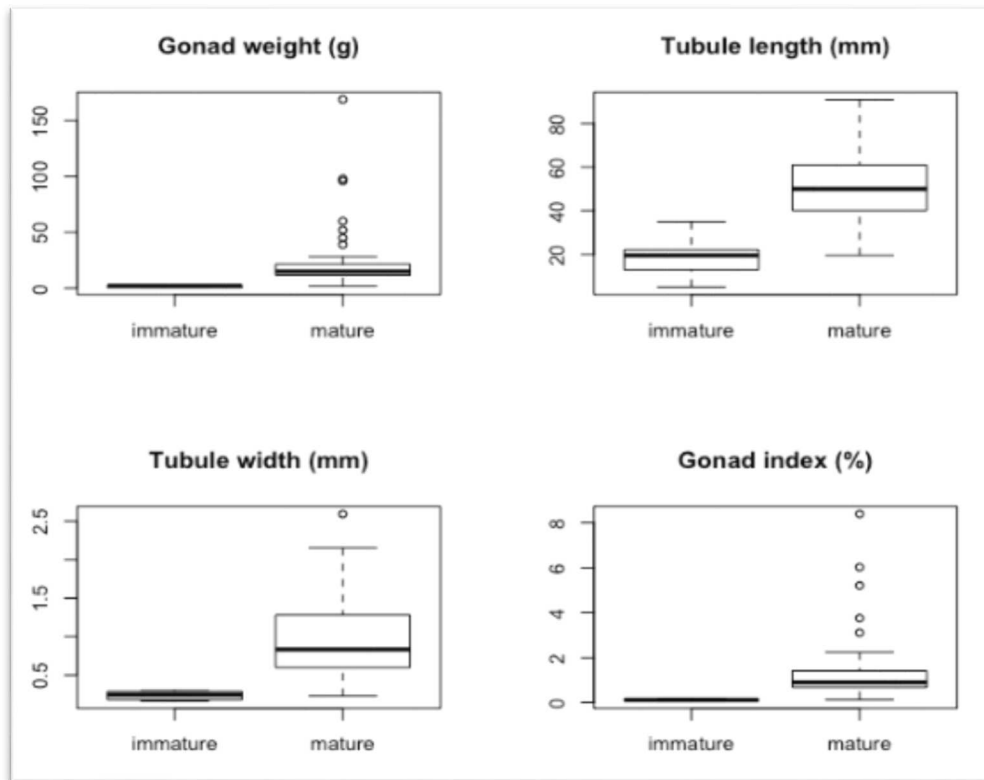


Figure 5: Distribution of gonad weight, tubule length, tubule width and gonad index function of mature and immature populations.

### *gonad index*

As expected, the GI increased from stage I&II to stage 4 and then decreased at the post-spawning stage to the same level as that of stage III. The similarity between stage III and V was still observable in figure 7. The gonad weight, tubule length and tubule width increased with the GI. For the total length, the trend curve increased slowly until a plateau between 300 and 350 mm.

The sex ratio was 1/1 (24 males, 24 females, and 19 sex undetermined).

### *size at first sexual maturity*

Several sexual stages were represented at the same period of time in both sampling sites. The most important part of the sample consisted in individuals at post-spawning and maturation stages (Figure 6). The distribution of sexual stages in the total sample indicated that the sampling had been done during the breeding season.

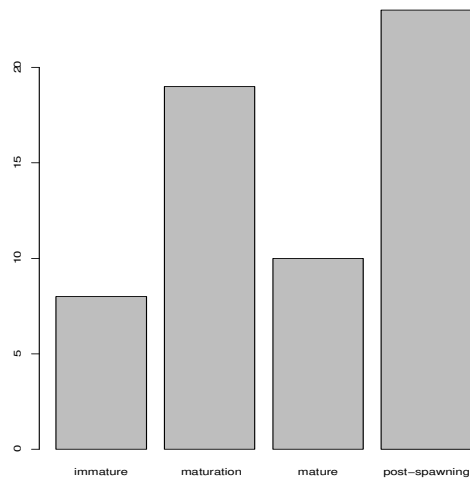


Figure 6 : Distribution of the different stages in the sample

However, the predominant sexual stage of specimens was different between Marie-Louise and Frégate sites, explaining the difficulty to find gonads in Amirantes. All stages were found at Frégate site while more pentard at stage I or II and without visible gonads were located at Amirantes site (Figure 8). Because most of those specimens were smaller than the sea cucumbers sampled at Frégate site, and because our observations provided evidence that the survey was conducted during the breeding season of Pentard, we hypothesized that the individuals without gonads were immature.



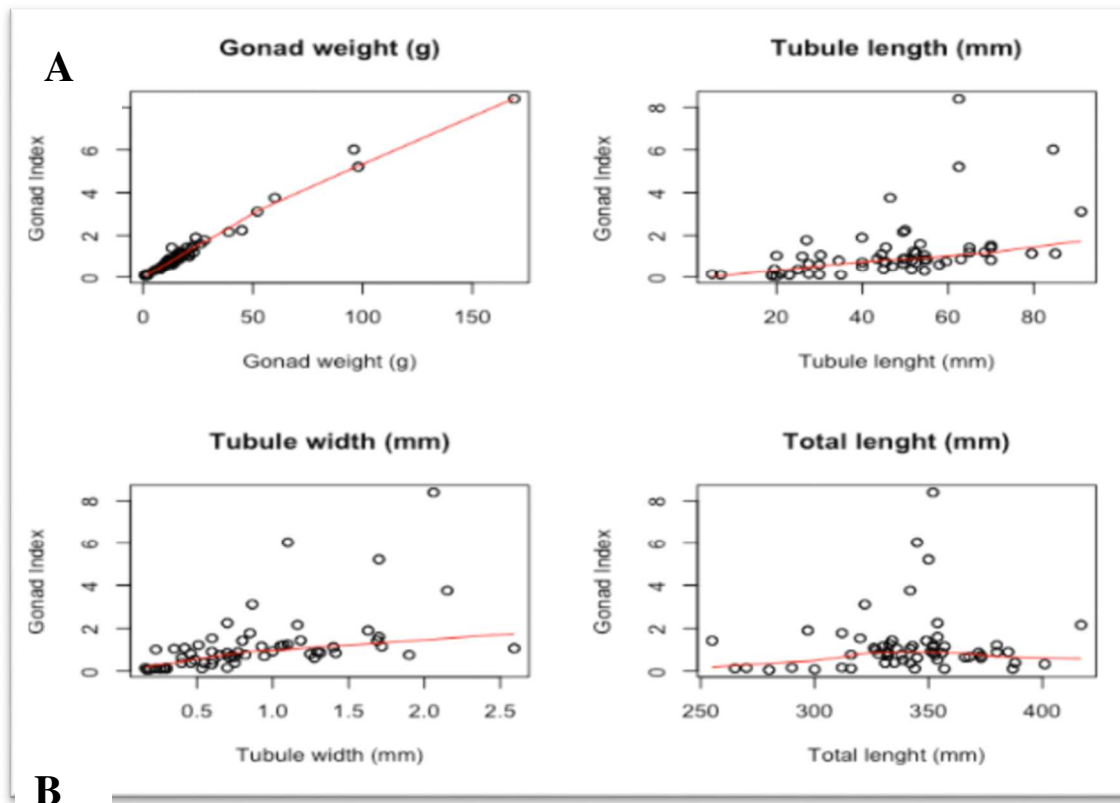


Figure 7 : A: Gonad index as a function of the gonad weight, tubule length, tubule width and total individual length. The red line shows the trend in GI.

In order to determine the size at sexual maturity, Both methods for estimating maturity size yield slightly different results. Based on cumulative frequency of mature individuals and logistic regression, the length at sexual maturity was estimated at 332 mm (figure 9) and XXX mm (95% confidence interval: 285-323 mm, figure 10), respectively.

To explain the relationship between those variables and to create a mathematic model, a logistic regression has also been used (Figure 10). This regression can monitor the degree of sexual maturity function of the size and give an estimation of the L50. The following equation curve has been used:

$$P = 1 / (1 + e^{-r(L-L_{50})})$$



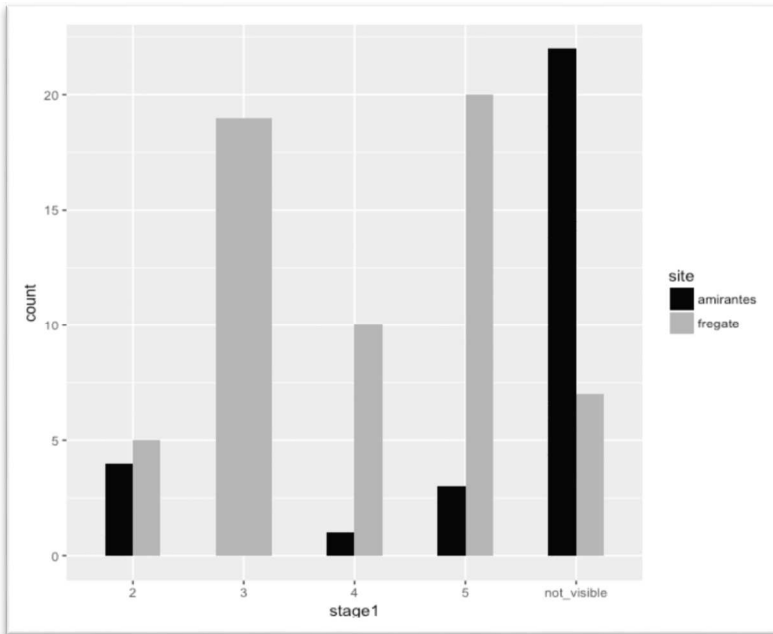


Figure 8: Number of individuals in each stage on the different sampling sites

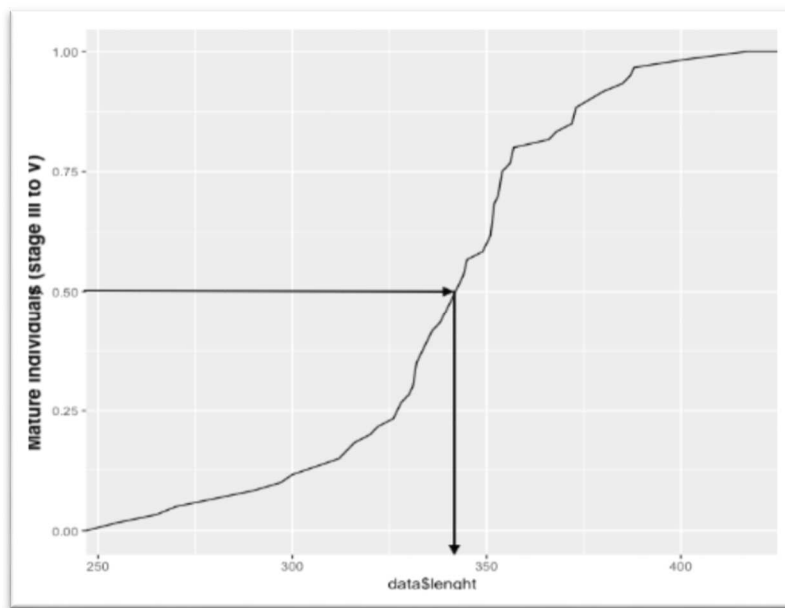


Figure 9: Size at first sexual maturity of pentard using the cumulative curve of percent of mature individuals. Y axis is "cumulative frequency" and X axis is length of mature individuals.

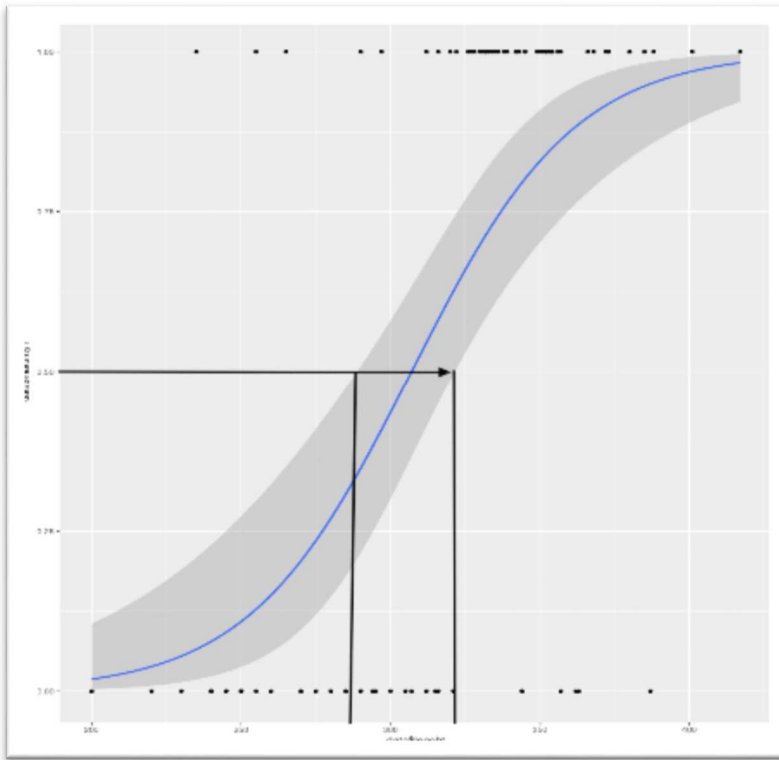


Figure 10: Size at first sexual maturity of pentard using a logistic regression with a mathematic model

## ***Discussion***

Though pentard is the most important holothurian species fished in Seychelles. Due to knowledge gaps on the reproductive biology of Pentard, any attempt to compare our results with other populations was difficult. Therefore, maturity size estimates were compared with those of other *Holothuria* testfish species in the Seychelles and with other *Aspidochirotida* species in other regions of the world.

The mean total length (317 mm) and the mean total weight (1462 g) of pentard are lower than the ones for *H. nobilis* (370 mm and 1878 g) and *H. fuscogilva* (401 mm and 2111 g) studied by Conand in New Caledonia (Conand 1981).

We observed that the size of pentard was different between the sample sites, although this result might have been affected by the low number of specimens sampled at the Amirantes site. It had been already reported that the morphology of animal may vary according to the environment (Conand & Muthiga 2007).

### *Gonad and gonad index*

The gonad of holothurian can also vary between sites, like for size for example (Sewell 1992; Hamel & Mercier 1996), as observed for *C. frondosa* along the Canadian coast (Hamel & Mercier 1996). It would be useful to explore this hypothesis in the Seychelles using a larger sample of Pentard, as this would generate different fishery management measures across sites. The distribution of gonad stages was also not uniform over the pentard populations of the sampling sites. The five stages were observed simultaneously, especially at Frégate site, as reported for other species. For instance Ramofafia and Byrne (2002) described that the gonad development of *H. scabra* was different across individuals within a single site.

A one-year study with monthly measures of the gonad index would help to determine the reproductive cycle of pentard more accurately, as that species may spawn at different periods. Previous studies have proven that *H. fuscogilva*, *T. ananas* spawn during summer while *H. nobilis* preferentially spawns during winter while *H. atra* spawned bi-annually in New Caledonia (Conand 1981, 1993, 2002).

The mean GI appeared larger for male than female pentard despite both sexes having similar average body weights (Table 2), in contrast to the previous observations on *H. nobilis* and *H. fuscogilva* (Conand 1981; Asha & Muthiga 2008; Muthiga & Kawaka 2009; Muthiga & Kawaka 2010; Parker and al 2018). However, the male GI was higher than the female GI in the *Cucumaria frondosa*, a dendrochirotid in Canada (Hamel & Mercier 1996).

The gonads weight of males was appeared as bigger than that of females (Table 1 and figure 11), but no difference in total weight was noticed. This could explain the difference between male and female GI and should be investigated in a next analysis with larger increased sample sizes and longer sampling periods.

The gonad resorption after the spawning season depends of the species. It is common for *H. fuscogilva* but very rare for *H. scabra* (Ramofafia & Byrne 2002, Muthiga and al 2009). In this study, the breeding season was hypothetized based on fishers' knowledge and the reproduction cycle of the *H. fuscogilva*, a genetically close species (Paulay G., pers. com.). the absence of gonad in Amirantes is supposed more linked of immaturity than gonad resorption.

The maturity of the gonads of pentard was similar to that of other holothuridae species described in Conand (1981, 1989) which defined a mean diameter of oocytes in stage V of 150  $\mu\text{m}$  comparable to 160  $\mu\text{m}$  for pentard. Gonad tubule length has been shown to correlate with sexual maturity (Muthiga and al 2009). Gonad tubule growth can therefore be used to monitor reproductive activity of sea cucumbers.

Parameters of gonad tubule follow the trend of the GI, indicating that they are good predictors of reproductive output in this species (Muthiga and al 2009). The mean tubule length (49.9 mm) of pentard was smaller than that of other species such as *H. fuscogilva*, that ranged between 79 and 88 mm in New Caledonia (Conand 1993) and 119 mm in Kenyan (Muthiga & Conand 2014) during spawning. Throughout the study, female tubules were wider than male tubules, which is a common pattern for many holothurians (Conand 1981, Navarro and al. 2012, Ghobadyan and al. 2012). However, tubules of males were slightly longer than those of females, as observed for other Aspidochirotida species such as *H. fuscogilva*, *H. nobilis*, and *H. scabra* (Conand 1993).

Despite a lack in robustness of the test in this study due to the small sample size, gonads displayed a sexual dimorphism (Figure 11). It had been already reported through other studies (Conand 1981, 1993; Navarro and al 2012) for some teatfish species.

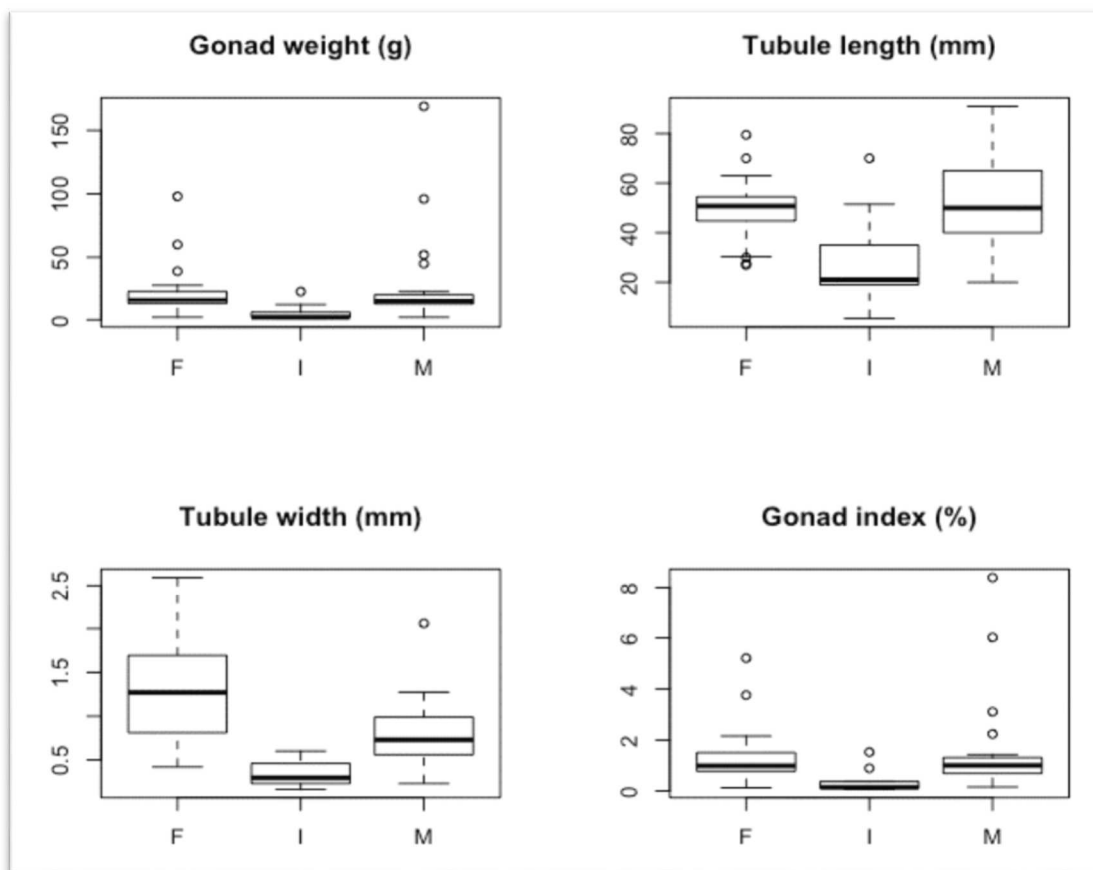


Figure 11: Distribution of gonad weight, tubule length, tubule width and gonad index  
F= female, M= male, I =undetermined

### *size at sexual maturity*

It is well known that a minimal body size is necessary before an individual becomes reproductive (Lawrence 1987). This size is necessary for fisheries management by helping to set the minimum harvest size which allow individuals to spawn before, in order to preserve the stock of pentard. The size at sexual maturity has been determined for other teatfish holothurians in New Caledonia, *H. fuscogilva* at 324 mm and for *M. nobilis* at 227 mm using a cumulative curve of mature individuals (Conand 1981).

In this study, both methods used to determine the size at sexual maturity led to a 2-cm difference in size estimates. The regression logistic is based on a predictive model constructed with binomial variable.

For pentard, the size at first sexual maturity found is about 332 mm with the cumulative curve and with the statistical method it lies between 285 and 323 mm. An extensive sampling may permit to have the same value and it should be test in a next study. As might be expected, the size at first sexual maturity is smaller for pentard than *H. fuscogilva* and *M. nobilis* according to its smaller total length.

### *The sea cucumber fishery*

Inadequate regulatory measures have contributed to poor resource status of most sea cucumber fisheries in the Indian Ocean (Ericksson and al 2015). In Seychelles for instance, the lack of biological knowledge on the biology of pentard makes it difficult to set an appropriate minimum catch size of that species. To date, no legal catch size of pentard has been established. Improving the fishery management is the best way to provide long term income for fishers.

According to Purcell and al (2017), introduction of a minimum catch size for pentard could lead to a significant increase in income from the fishery given that large sea cucumbers are given a higher price on the Chinese market. However, to date, fishers have usually been paid per unit of sea cucumber fished in Seychelles rather than per kg (pers. obs.), which means that they have had little incentive to search for large specimens..

The mean dried size of pentard relieved on the Chinese market ranges between 14 and 25cm, although there is no information about the fishing sites of those products (Purcell and al 2017). Using available conversion ratio (Purcell and al 2009, 2017; Aumeeruddy & Conand 2007), who had assessed the difference between of fresh and dried size of pentard and other teatfish, the estimated live size of those sea cucumber would range between 23 and 42 cm, which

includes non mature specimens. This illustrates the need for introducing a minimum catch size in Seychelles.

Fecundity is another important tool in the fishery management since, besides the first sexual maturity, sea cucumber must reproduce to increase or maintain the stock. Future studies are encouraged to focus on the fecundity per size to estimate the ideal size to capture.

Using the DNA fingerprint of *H. nobilis*, Uthicke and al (2004) shown that, for *H. (Microthele) nobilis* indicated that productivity of holothurian stock is low, and a long time will be needed to the stock to recovery a consequent population. Conand and al (2004) found the same conclusion, fishing reduced the stock without appreciable replenishment. It is a considerable factor who should be taken in account to react to the intensive pentard fishery in Seychelles.

## **Conclusion**

This study on the pentard has provided the first data set on the reproductive biology of this species in Seychelles. The size at maturity was determined using both cumulative curve and logistic regression. Those models suggested two sizes close, yet different size estimates. he minimum catch size according to the size at first sexual maturity whichever is smaller, could be 285 mm.

Although the results should be confirmed by additional data from other fishing sites and/or larger samples, those estimates are useful to introduce a minimum catch size of pentard in the Seychelles. This would likely contribute to the sustainable exploitation of the resources.

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## **Abstract**

The intensive fishing of sea cucumber in Seychelles, mainly *Holothuria* (*Microthele*) *pentard*, has led to the overexploitation of the resources in the last decade. The biology of this species (locally named “pentard”) is largely unknown. In order to improve the management of this fishery, this study assessed the size at first sexual maturity of pentard. The reproductive biology of this species was studied in the Mahé plateau and Les Amirantes plateau. A combination of macro- and microscopic analysis of gonads and a description of tubules was performed. Two different mathematics methods (a cumulative curve of mature individuals and a logistic regression model) were used to estimate size at maturity. The population of pentard at Les Amirantes site was smaller in size than at Frégate site. According to fishers’ knowledge and the reproductive cycle of closest species as *H. fuscogilva*, pentard had a summer spawning. Females tubules were wider and smaller than males tubules testifying a sexual dimorphism between them. The size at sexual maturity found with the two methods was closed but different. As a precaution, it was decided that the smallest size was retained: 285 mm from the mouth to the anus. These results could be useful to introduce a minimal catch size and thus maintain the stock and hopefully increase the population of *Holothuria pentard*.

## **Keywords**

pentard, holothuria, sexual maturity, reproduction, Seychelles