Size at sexual maturity of the flower teatfish *Holothuria* (*Microthele*) sp. in the Seychelles

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Abstract

The intensive harvesting of sea cucumbers in the Seychelles over the last decade has raised concerns about the sustainability of this resource. The flower teatfish *Holothuria (Microthele)* sp. (locally called pentard) is one of the main target species, representing about two-thirds of annual sea cucumber catches. The biology of this species is largely unknown. To inform fishery management, the reproductive biology of the species was studied on the Mahé Plateau and the Amirantes Plateau during the 2018 northwest monsoon season. A macroscopic analysis of the gonads, and a description of the tubules were performed on 63 sea cucumbers. We observed that the population at the Amirantes Plateau was smaller in size than those from the Mahé plateau, although that difference was not statistically significant. Female tubules were wider and shorter than male tubules, showing there is sexual dimorphism in this species. The size at sexual maturity of flower teatfish was estimated at 30.3 cm (95% confidence interval: 28.5–32.3 cm) through logistical regression. Based on this result, a minimal harvest size of 31 cm may be recommended for this species as a conservative measure in the Seychelles.

Introduction

Holothurians have been fished in the Seychelle Islands of the southwest Indian Ocean for hundreds of years (Aumeeruddy and Conand 2008). They are mostly collected by divers using scuba gear at depths of around 15 to 45 m. They are further processed and dried and exported to Asian markets as beche-de-mer. Globally, holothurian resources have increasingly been overexploited since the 1990s, due to the rising demand by the Chinese market. Up until the end of the 20th century, sea cucumber harvesting in the Seychelles was an open access fishery, without any management control due to the low economic value of the fishery. However, the Seychelles Fishing Authority (SFA), the governing body responsible for fisheries management, introduced a national management plan in 2005 to guide the management of the fishery as a response to the increase in fishing pressure and annual catches (Conand and Muthiga 2007). The teatfish group within the genus *Holothuria* (white teatfish *H. fuscogilva,* black teatfish *H. nobilis,* and flower teatfish *H.* (*Microthele*) sp. (locally known as pentard, Fig. 1) has been heavily targeted in the Seychelles in the 2000s because they are among the most valuable species on the world market (Purcell et al. 2012, 2017, 2018; Diassanayake and Gunnar 2010). With approximately 150,000–300,000 individuals harvested per year, the flower teatfish has been the main commercial species targeted in the Seychelles since 2006, followed by prickly redfish

Figure 1. Flower teatfish – *Holothuria* (*Microthele*) sp. (locally called pentard) – from the Seychelles.

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Thelenota ananas (15,000–72,000 individuals per year) and white teatfish (37,000–127,000 individuals per year) (Aumeerudy and Conand 2008; Léopold and Govinden 2018). The increasing demand for these species, driven by their increasing commercial value, has progressively led to their potential overexploitation as observed by the declining trend in catches over the last five years.

The flower teatfish has been observed in the Seychelles, Comoros, Tanzania, Madagascar, Sri Lanka and the Maldives (Purcell et al. 2012). However, to date, that species has not yet been formally described, although this work is ongoing (G. Paulay, Curator, Florida Museum of Natural History, pers. comm.). Information on the ecology of flower teatfish is poor, which makes stock assessment difficult and the design of suitable management or conservation measures uncertain.

To address this gap, this study aimed to improve our knowledge of the reproductive behavior of flower teatfish. Specifically, we conducted a biological survey to determine its size at first sexual maturity. The present work was performed as part of a larger research programme whose overall purpose was to strengthen the sustainability of the sea cucumber fishery in the Seychelles through adaptive co-management (Léopold and Govinden 2018).

Materials and methods

Survey area and sampling

The survey was carried out on board SFA's research vessel at two sites: one close to Fregate Island on the Mahé Plateau and the other close to Marie-Louise Island on the Amirantes Plateau, in February and March 2018, respectively. According to fishers, flower teatfish spawns during the monsoon period (October–March), which is also the spawning period of the related species white teatfish. Eight fishers, working on two commercial fishing vessels, volunteered to participate in the survey and facilitate the collection of sea cucumbers. This served to also improve awareness of the research program among fishers. The fishers manually and opportunistically collected flower teatfish specimens while scuba diving at depths ranging between 25 m and 30 m depth at different locations in both survey sites.

The following observations were made by the scientific team on the fishing boat and on all specimens immediately after capture. Total body length (TL) was measured from mouth to anus to the nearest 5 mm with a metric tape and weighed to the nearest 1 g using an electronic balance without an anti-rolling system, and this likely affected the accuracy of weight measurements. Fishers then made a cut on the dorsal side of each specimen (Fig. 2). The entire gonad was removed, weighed to the nearest 1 g, and stored in individual 50-mL tubes filled with 10% formalin. After removing the internal organs and excess coelomic fluid, the gutted body was weighed to the nearest 1 g.

Figure 2. A fisher making a dorsal cut on a freshly harvested flower teatfish in order to remove the viscera.

The sexual stage was determined using the procedure defined by Conand (1981). Firstly, the gonad index (GI) was calculated as follows:

$$GI = Wgonad \times 100 / Wtot$$

where Wgonad = gonad weight (g) and Wtot = total weight (g). GI was expected to be high when gonads were mature.

Secondly, a macroscopic description of the gonads was conducted at SFA's laboratory. Although the sexes are separated in this species, there is no morphological sexual dimorphism (Ghobadyan et al. 2012). Each gonad sample was first placed in a Petri dish. The approximate longest tubule was measured with a measuring tape to the nearest 0.1 mm. The average width of the tubules was measured to the nearest 0.1 mm on digital pictures using a trinocular microscope (x 1.8 zoom) using the Motic Image Plus 2.0 software. Then the tubules were cut using a scalpel. Their content was placed within a concave blade to determine the sex and maturity stage based on the description of maturity stages by Conand (1981), whereby five maturity stages were established according to morphological parameters of the gonad tubules: immature (I), resting (II), growing (III), mature (IV) and post-spawning (V). Holothurians were defined as mature between stages III and V. For females, digital pictures were used to measure the diameter of approximately 10 oocytes to the nearest 0.1 mm using the same procedure as described above.

Data analysis

The total body weight and gonadal morphological parameters were compared across maturity stages using the Kruskal Wallis non-parametric test. The same test was used to compare the weight and size of specimens between the two survey sites.

Finally, the size at first sexual maturity (L50) of flower teatfish was defined as the length at which the gonads of 50% of individuals were mature (Conand 1981; Navarro et al. 2012; Muthiga and Conand 2014). To estimate the L50 and its 95% confidence interval, the proportion of mature individuals at different size intervals was modelled using a logistic regression as defined by the following equation:

$$P = \frac{1}{1 + e^{-r\,(L_t - L_{50})}}$$

where P = proportion of mature individuals, L_t = total length (cm), L_{50} = length at first maturity (cm), and r = model's parameter.

Statistical data analysis was performed using R v 1.1.442.

Results

In this study, 92 flower teatfish were sampled (31 from Mahé Plateau and 61 from Amirantes Plateau), and 54 gonads (88.5% of the sea cucumbers collected) were collected from the Mahé Plateau and 9 gonads (29%) from the Amirantes Plateau.

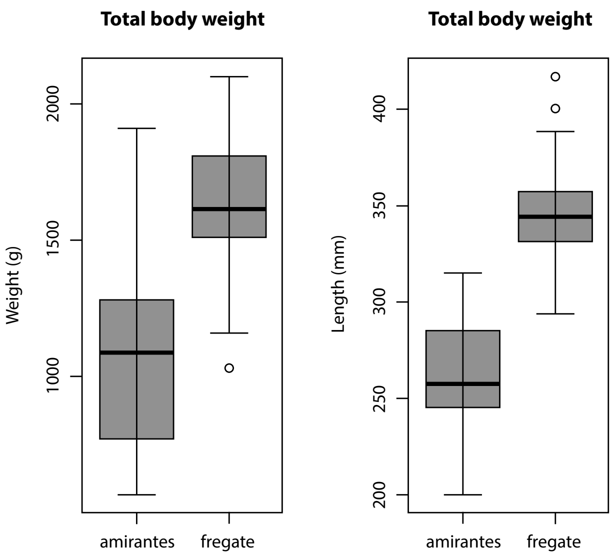

Figure 3. Differences in total weight (g) and length (mm) between the two sampling sites (Amirantes Plateau and Fregate Island on the Mahé Plateau). Lower and upper levels of the boxplot: lower and higher quartiles; black line: median. Dotted lines: lower and upper whiskers.

On the Amirantes plateau, the length of the individuals ranged from 200 mm to 315 mm (mean 263 mm, SD 26 mm) and the total body weight ranged from 563 g to 2153 g (mean 1,068 g, SD 38 g). The length and total weight at Mahé Plateau and Amirantes Plateau are shown in Figure 3. The difference in weight and length between sites was not significant: Kruskal Wallis test, p(weight)=0.36, p(length)=0.06.

Gonad morphology

The characteristics of the five maturity stages were described (Figs. 4 and 5):

- Stage I and II: Because we did not know the size at first sexual maturity, it was not possible to distinguish stage I (immature) from stage II (resting). At both stages, the sex could not be determined.
- Stage III: Maturation started, with growing gonads and exposed and branching tubules evident. Small oocytes were visible, while only unclear liquid was observed within the gonads of the males.
- Stage IV: Gonads were fully grown and mature. Female tubules were filled with oocytes and male tubules showed several bulges filled with sperm.
- Stage V: Although gonads were broadly similar to those at stage III, they displayed a smaller gonad size and dropped tubules typical of the post-spawning stage. Some atretic oocytes were also present.

The gonad weight, tubule length, tubule width and gonad index measured showed significant differences between males and females and between mature and immature individuals (Table 1).

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

Gonad index

As expected, GI increased from an average of 0.60 (immature specimens) up to 1.45 (mature specimens). Gonad weight, tubule length and tubule width increased with GI (Fig. 6). GI increased with the total body length up to about 330 mm total length (Fig. 6D).

Size at first sexual maturity

Several sexual stages were observed at the same period in both sampling sites. The most important part of the sample from Mahé Plateau consisted of individuals at post-spawning (stage V) and maturation (stage III) stages. The distribution of sexual stages in the whole sample indicated that the

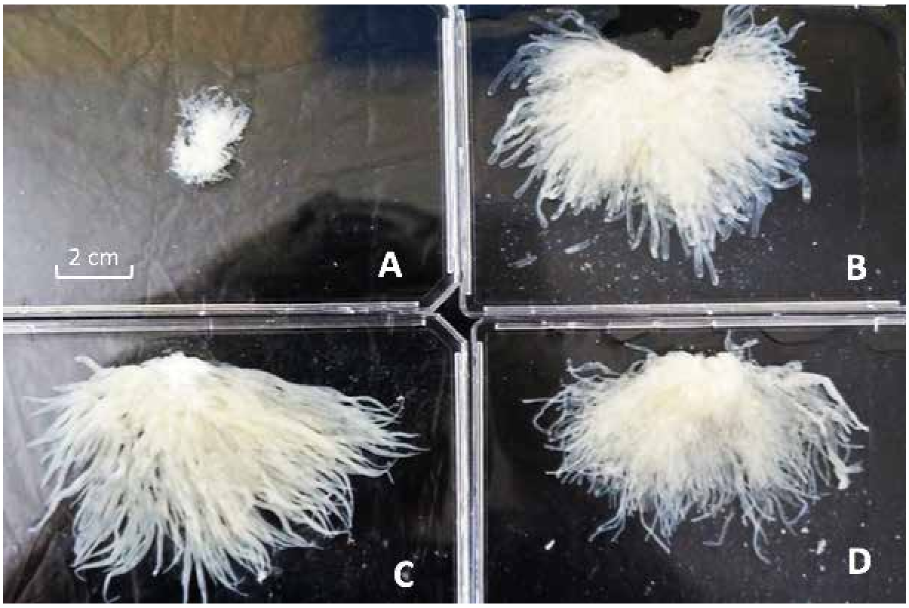

Figure 4. Female gonads of flower teatfish. A: Immature (stage I–II), B: maturation (stage III), C: mature (stage IV), D: post-spawning (stage V).

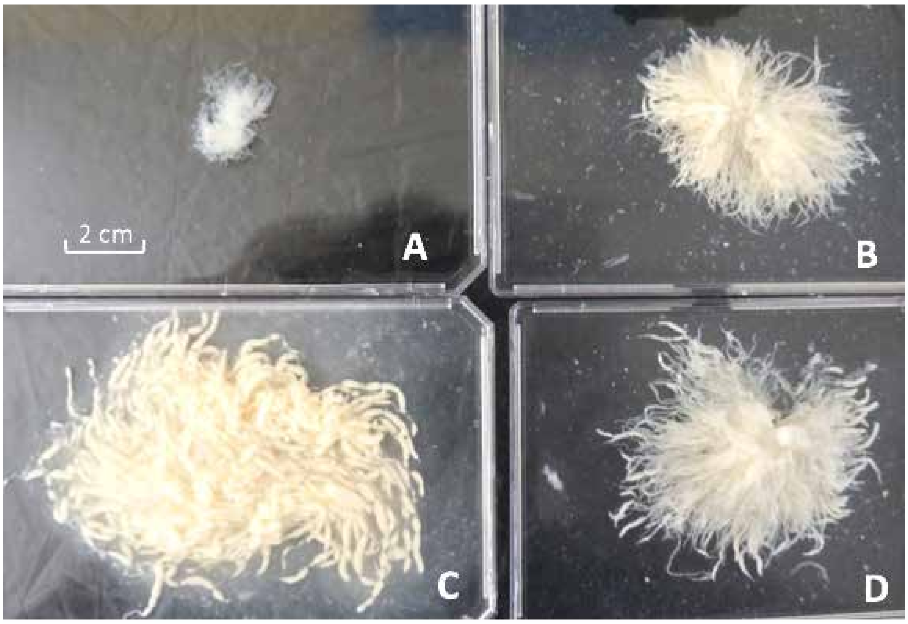

Figure 5. Male gonads of flower teatfish. A: Immature (stage I–II), B: maturation (stage III), C: mature (stage IV), D: post-spawning (stage V).

Table 1. Description of the gonads of immature and mature flower teatfish specimens: gonad weight, tubule length, tubule width and gonad index, with the p-value, mean (SD). The p-value of the Kruskal-Wallis test is indicated (see Methods section for more details).

	Gonad weight (g)	Tubule length (mm)	Tubule width (mm)	Gonad index
Immature	1.56 (0.53)	18.62 (9.38)	0.24 (0.05)	0.58 (1.46)
Mature	23.53 (27.87)	50.27 (16.69)	0.99 (0.52)	1.45 (1.55)
p-value	<0.001	<0.001	<0.001	<0.001

Table 2. Number of flower teatfish specimens at each sexual maturation stage sampled at both survey sites in the Seychelles.

	Immature (I)	Maturation (III)	Mature (IV)	Post-spawning (V)	Non detected gonad
Amirantes	5	0	1	3	23
Mahé	5	19	10	20	8
Total	10	19	11	23	31

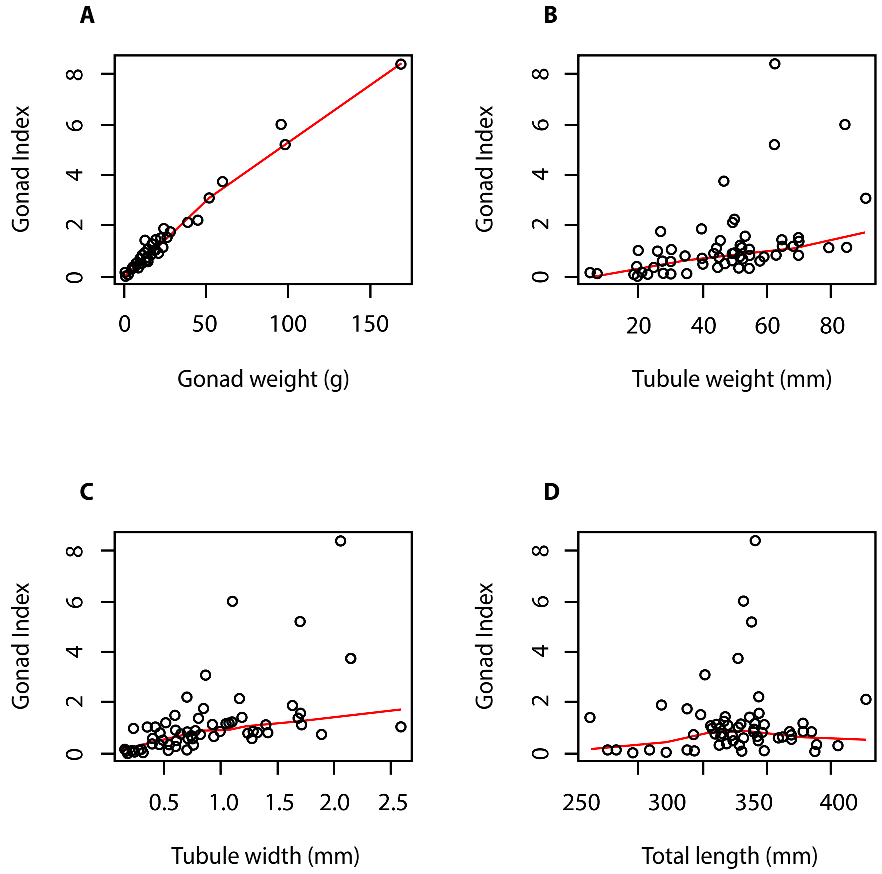

Figure 6. Gonad index as a function of gonad weight (A), tubule length (B), tubule width (C) and total individual length (D). Red lines show trends.

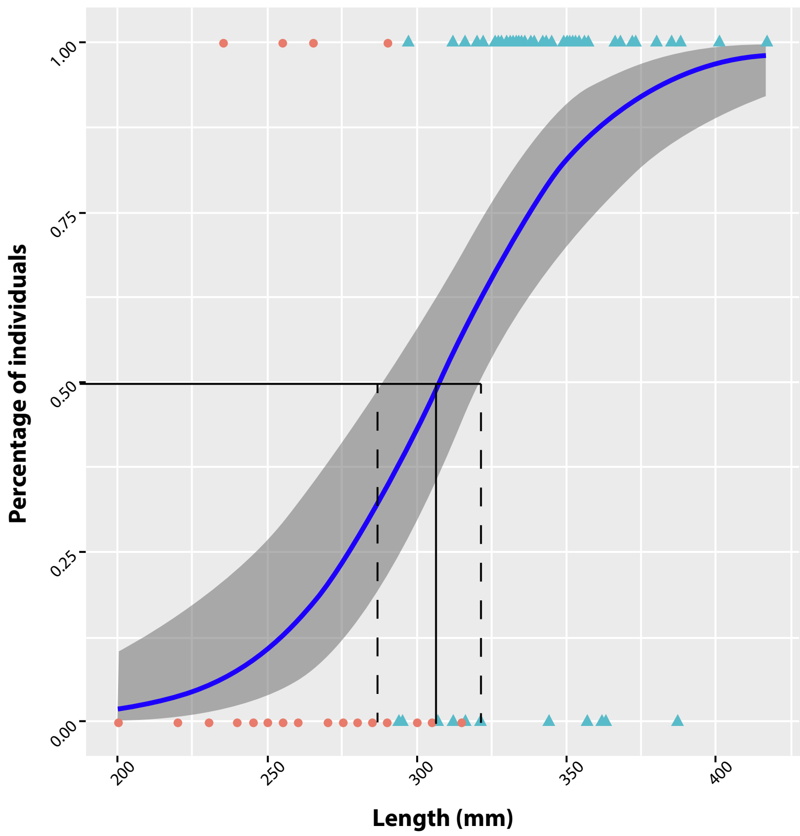

Figure 7. Proportion of mature flower teatfish as a function of individual length (n = 92). The logistic regression and the L_{50} (i.e. size at first sexual maturity) are shown. Red dots represent sea cucumbers from Amirantes Plateau, blue dots represent sea cucumber from Fregate Island on Mahé Plateau.

sampling occurred during the breeding season of this species. The predominant sexual stage of specimens was different between the two sites. Although all stages were found at Mahé Plateau, stages I and II were the ones most commonly observed at Amirantes Plateau, including those specimens for which no gonads were detected during onboard observations. Indeed, because most of those specimens were smaller than the ones from Mahé Plateau, and because our observations provided evidence that the survey was conducted during the breeding season of flower teatfish, we hypothesised that the individuals without gonads were immature. Based on the logistic regression, the length at sexual maturity was estimated at 303 mm (95% confidence interval: 285–323 mm) (Fig. 7).

Discussion

Reproductive biology of flower teatfish

This is the first study examining the reproductive biology of flower teatfish. It provides the first estimate of size at first sexual maturity for this species. The size at first sexual maturity had been determined for other teatfish holothurians in New Caledonia, such as white teatfish (324 mm) and black teatfish (227 mm), using cumulative curves (Conand 1981).

The description of maturity stages of flower teatfish was similar to other teatfish species in the family Holothuridae, especially the teatfish described in Conand (1981, 1989). We found that the distribution of gonad stages among samples was not uniform between the sampling sites. The five stages were observed simultaneously (although at varying rates) at Mahé Plateau, whereas no stage III was reported at Amirantes Plateau. Such a pattern was observed in other sea cucumber species. For instance Ramofafia and Byrne (2002) described that the gonad development of *H. scabra* was different across individuals within a single site in Salomon Islands. A one-year study with monthly observations would help to determine the reproductive cycle of flower teatfish more accurately as the species may spawn at different periods, which could explain the occurrence of the various sexual stages during our survey. Previous studies in New Caledonia have proven that white teatfish and prickly redfish spawn from December to February while black teatfish preferentially spawns from July to November (Conand 1981, 1993).

The presence or absence of gonads at the same period could also be linked with gonad resorption. This process was observed for white teatfish after the spawning season (Ramofafia and Byrne 2002; Muthiga and Conand 2014). In this study, the breeding season was hypothesised based on fishers' knowledge and the reproduction cycle of white teatfish, a genetically close species (G. Paulay, pers. comm.). The absence of gonads at the Amirantes site was interpreted as immaturity rather than gonad resorption because the specimens were smaller at Amirantes. Indeed, we observed that the size of flower teatfish was different between the sample sites, although this result was not statistically validated, likely due to the low number of specimens sampled at Amirantes Plateau. It has already been reported that sea cucumber morphology may vary according to environmental conditions and fishing pressure (Conand and Muthiga 2007). Our results should, therefore, be confirmed by using larger sample sizes collected in different habitats, and evaluating fishing pressure across those sites. Gonad sizes of holothurians can also spatially vary, as observed for *Cucumaria frondosa* along the Canadian coast (Hamel and Mercier 1996). It would be useful to explore this hypothesis in the Seychelles.

Mean GI appeared greater for male flower teatfish than for females, despite both sexes having similar average body weights. This is in contrast to observations of black teatfish and white teatfish in Kenya and New Caledonia which showed the opposite (Conand 1981; Muthiga and Kawaka 2009). This difference was very likely due to higher gonad weights for males than for females because no difference in total body weight was noticed. It should be further investigated using larger sample sizes and longer sampling periods.

Gonad tubule length was correlated to sexual maturity as observed by other authors (e.g. Muthiga and Conand 2014). The mean tubule length of flower teatfish during spawning (49.9 mm) was smaller than that of other species such as white teatfish, which ranges between 79 mm and 88 mm in New Caledonia (Conand 1993), and 119 mm in Kenya (Muthiga and Conand 2014). Gonad tubule growth can, therefore, be used to monitor the reproductive activity of sea cucumbers. Throughout the study, female tubules were wider than male tubules, which is a common pattern for many holothurians (Conand 1981; Navarro et al. 2012; Ghobadyan et al. 2012). However, male tubules were slightly longer than those of females, as observed for other aspidochirotida species such as white teatfish, black teatfish and sandfish (*H. scabra*) (Conand 1993).

Despite a lack of statistical robustness in this study, due to the small sample size and the variability of observations, gonads displayed a clear sexual dimorphism. Such a dimorphism has been reported for teatfish species in other studies (Conand 1981, 1993; Navarro et al. 2012).

Management implications

Inadequate regulatory measures have contributed to the poor resource status of most sea cucumber fisheries in the Indian Ocean (Eriksson et al 2015). In the Seychelles, for instance, the lack of knowledge on the biology of flower teatfish prevented the introduction of an appropriate minimum catch size limit for that species, which may be a relevant management recommendation for preserving resource biomass. Based on our estimate of the size at maturity of flower teatfish, a minimum catch size of 31 cm may be suitable for that species in the Seychelles, although the results should be confirmed by additional data from other fishing sites and/or larger samples.

The potential impact of such a rule is currently unknown given that there are no statistics on the size structure of sea cucumber catches in Seychelles. Nevertheless, some indications may be derived from Purcell et al. (2017) who reported that the mean dried size of flower teatfish recorded on the Chinese market ranged between 14 cm and 25 cm, although there was no information about the fishing sites from where these products came from. Using the available conversion ratio (Aumeeruddy and Conand 2007; Purcell et al. 2009, 2017), the estimated live size of these sea cucumber would range between 23 cm and 42 cm, which includes non-mature specimens. This highlights the need for introducing a minimum catch size in the Seychelles.

To date, fishers in the Seychelles have usually been paid per unit of sea cucumber landed rather than per kilogram (pers. obs.), which provides little incentive to search for large specimens of commercial species. The establishment of a minimum catch size could, therefore, modify catch rates and, consequently, the landed value of the fishery. According to Purcell et al. (2017), the introduction of a minimum catch size for flower teatfish 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. Using the above conversion rates for instance, specimens of 23–26 cm and 36–39 cm in length would be priced USD 5–17 and USD 54–66 per piece, respectively.

In conclusion, this study provides the first data on the reproductive biology of flower teatfish in the world. The results provide guidance for further research investigation and fishery management.

Acknowledgments

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