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Notes on the Reproductive Traits of *Holothuria fuscogilva* Cherbonnier, 1980 from Laguindingan, Misamis Oriental, Philippines

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ABSTRACT

Reproductive traits of white teatfish *Holothuria fuscogilva* from Laguindingan, Misamis Oriental were investigated from February to July 2020. Gonad samples from 62 adult individuals weighing 1,240g to 5,800g were collected and examined macroscopically and microscopically to determine gonad morphology, sex ratio, gonad maturity, and gonad index. Gonads were composed of numerous branched tubules of varying lengths and frequency of bifurcations arising from the gonad basis. There was no significant variation in the ratio of male to female white teatfish ($x^2=2.32$, $\alpha=0.05$) and were mostly mature in May (weighing 1,750g–4,000g in males and 2,600g-3,900g in females) with relatively high gonad output. Information on gonad maturation is useful in the development of a successful breeding technology for this economically important sea cucumber species.

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gamete release; conservation biology; gonad index; gonad morphology; sex ratio; white teatfish

INTRODUCTION

The white teatfish *Holothuria fuscogilva* Cherbonnier, 1980 is a large, tropical sea cucumber species that commonly occur on seagrass beds and coral reef slopes in the Indo-Pacific region and one of the highest valued sea cucumbers in the world, primarily for the trepang industry and as traditional medicine, and aphrodisiacs (Conand and Byrne et al., 1993; Toral- Granda, 2007). As a result, the high demand for trepang has led to massive harvesting and overexploitation. Restocking of *H. fuscogilva* wild populations, and establishing minimum size limits, closed seasons, and bag limits are likely to be options to restore and enhance wild stocks and/or bail out the resource from imminent danger of extinction (Battaglene and Bell, 19999; Conand et al., 2014). Further, the inclusion of *H. fuscogilva* as species of high concern for conservation in Appendix II of CITES along with other teatfish species *Holothuria (Microthele) nobilis* (Selenka, 1867) and *Holothuria (Microthele) whitmaei* Bell, 1887 is a significant measure to mitigate trading and overexploitation.

Much of our knowledge regarding the reproductive biology of the white teatfish is derived from limited pioneering studies, such as those of Conand (1981); Reichenbach (1999); Ramofafia et al. (2000); and Muthiga and Kawaka (2009), which provided various detailed descriptions of the reproductive behavior and timing of this commercially important holothurian species. In the Philippines, a CHED-funded project by De Guzman and Quiñones in 2011-2012 reported viable populations of H. fuscogilva in Capayas Island Marine Sanctuary of Lopez Jaena, Misamis Occidental. This research served as a catalyst for the DOST-GIA-funded project in 2014-2016 on the "Development of Captive Breeding and Hatchery Technology for the White Teatfish, *Holothuria fuscogilva* (Cherbonnier 1980)" in Lopez Jaena, Misamis Occidental and Laguindingan, Misamis Oriental by Lumasag and colleagues. Although the project has successfully produced juveniles from a single spawning of white teatfish broodstock from Lopez Jaena, Misamis Occidental, further research on the ecology and biology of *H. fuscogilva* was recommended. Together, these projects provided a springboard for the current DOST-PCAARRD-funded program establishing MSU at Naawan Sea Cucumber Research and Development Center (SCRDC) as Niche Center in the Regions for R&D (NICER).

The presence of adult white teatfish individuals and the lack of scientific knowledge and resource management for the species were considered in selecting the Municipality of Laguindingan as one of the monitoring sites of the NICER program. A significant output of the program is information on the reproductive biology of the white teatfish in Laguindingan, such as gonad morphology, sex ratio, gonad maturity, and gonad index which are useful in crafting management and conservation measures for this economically important sea cucumber species.

MATERIALS AND METHODS

Study area

The samples were collected from the deep waters (average depth of 20 meters) of Tubajon, Laguindingan, Misamis Oriental (8°35'N and 124°27'E; Fig. 1). Adult *H. fuscogilva* are specifically present in unprotected reef locations and are not actively collected nor traded. Underwater temperature at the depth of 31 meters on March 2021 was recorded to reach 25°C. Although previous work of Lumasag et al. (2017) considered the area as one of the collection sites for the *H. fuscogilva* broodstock, there remains a lack of comprehensive research on the ecology and biology of the species which are otherwise necessary because of its known potential in mariculture and in alleviating a lot of people who are dependent on this high-value species as a source of livelihood (Purcell et al., 2012). Finally, there is no existing management strategy focused on the sea cucumber resources of the municipality (LGU-Laguindingan Municipal Agriculturist, pers. comm.).

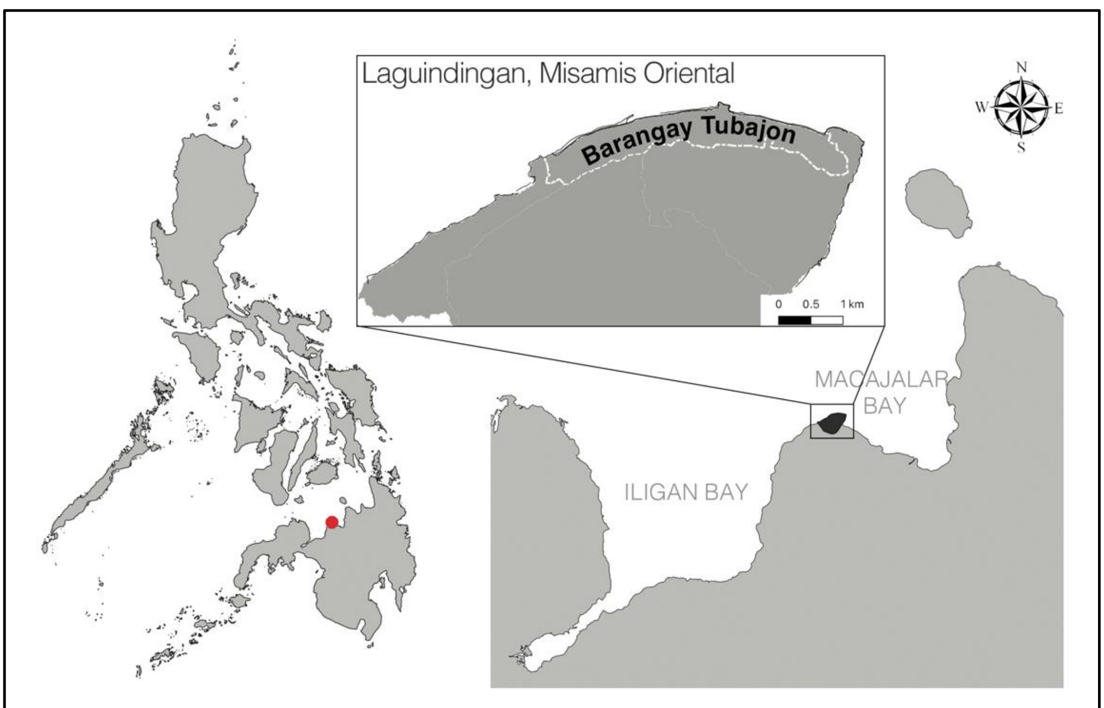

Figure 1. Map showing the coastal barangay of Tubajon, Laguindingan, Misamis Oriental within Macajalar Bay in the southern Philippines.

Sample Collection

Sixty-two (62) male and female live white teatfish weighing 1,250g to 5,550g, and 1,600g to 5,800g, respectively, were collected by local fishers from February to July 2020. Pursuant to Republic Act No. 9147, a Prior-Informed-Consent (PIC) certificate was secured from LGU-Laguindingan for the collection of specimens. These are decisive groundwork considering the species' threatened status and inclusion in CITES Appendix II (Di Simone et al., 2020). Figure 2 shows an adult white teatfish with its prominent body coloration and lateral teats. Since *H. fuscogilva* is not sexually dimorphic, gonad examination was the only way so far to determine the sex of individual samples. With this, gonad samples were obtained by making a 2-4cm incision near the anterior region in the ventral part (trivium) of the organism while applying slight pressure at the posterior region (Reichenbach, 1999). Gonads were then weighed individually and preserved in 10% formalin in seawater. Smears were also prepared from gonad samples and viewed under a microscope to confirm the sex previously assigned to sampled individuals (Ramofafia et al., 2000).

Analyses of gonad tubules

Ten tubules were selected from each preserved gonad sample for macroscopic and microscopic examination, noting their morphological characteristics such as color, tubule length, diameter, frequency of bifurcation, and branching. Gonad tubule length was measured from the gonad basis to the tip of the tubule (Muthiga and Kawaka, 2009)

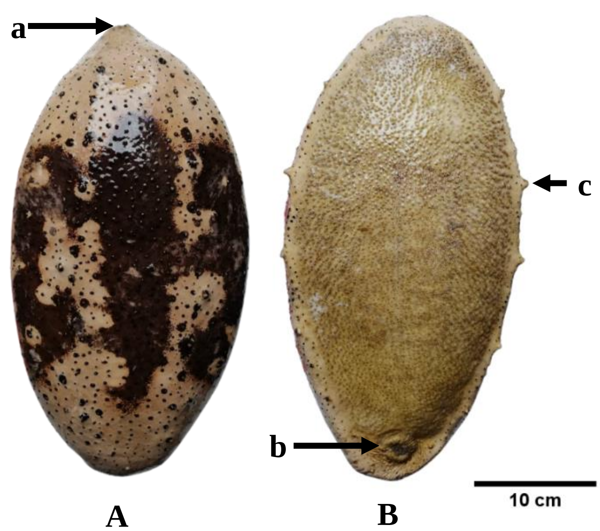

Figure 2. (A) Dorsal and (B) ventral sides of an adult *Holothuria fuscogilva* showing the anus (a), mouth (b), and lateral teats (c).

using a tape measure. Gonad basis is a structure where individual gonad tubules are collectively connected which is then connected to the gonoduct (Ramofafia et al., 2000; Hamel and Mercier, 2007). Tubule diameter was measured microscopically under a 4x objective. Preserved gonads were examined macroscopically to determine the gonadal maturity stages of each gonad sample following the five-stage maturity scale of Ramofafia et al. (2000).

Data analyses

The computed sex ratios for each sampling month were tested for significant difference from the unity which refers to the theoretical sex ratio 1:1 using the Chi-square test, while the level of synchrony between sexes was determined by a correlation between the mean monthly gonad index of males and females. Gonad indices were calculated using the formula: gonad wet weight/body wet weight x 100. All statistical tests were done using PaST version 4.03 software (Hammer et al., 2001) at a 95% confidence level.

RESULTS AND DISCUSSION

Gonad morphology

Generally, male and female gonads are characterized by numerous branched tubules arising from the gonad basis (Fig. 3a) with varying lengths and frequency of bifurcation (Fig. 3b, c). As observed, male and female gonads showed distinct characteristics depending on the maturity stage. Male gonad tubules have a beaded appearance even at the growing stage (Stage II) (Fig. 3d) although not distinct but become more conspicuous as it approaches maturity (Stage III) (Fig. 3e) while spent (Stage V) male gonads have shrunken and wrinkled tubules (Fig. 3f). On the other hand, mature female gonads have translucent tubules to the extent that the oocytes can be seen through the tubule wall (Fig. 3g). Female gonads identified as partly- spawned (Stage IV) resembles a mature gonad but with the presence of phagocytes in the tubule along with mature oocytes (Fig. 3h) and few tubules possessed a yellowish grain-like structure (Fig. 3i). Spent female gonads have tubules that appeared shrunken and wrinkled with a greatly reduced concentration of oocytes. The stages of gonad development based on the tubule characteristics are detailed in Table 1, although the confirmation of its maturity stage needs to be done via histology which is still an ongoing aspect of the current study.

The observed morphology of the male and female gonads of *H. fuscogilva* in the study is similar to those observed by Ramofafia et al. (2000) in the Solomon Islands while the presence of different tubule length groups is akin to those observed in *Holothuria whitmaei* (Shiell and Uthicke, 2006) in the Indian and Pacific regions of Australia. Variation in lengths, diameters, and frequency of bifurcations between maturity stages as shown in Table 1 can be attributed to the fact that gonad tubules increase in size and in the number of tubule branches to increase the volume of the gonads as gametes developed (Ramofafia et al., 2000; Muthiga and Kawaka, 2009). The marked variation in tubule length can be an indication that *H. fuscogilva* follows the Tubule Recruitment Model (TRM) wherein tubules are progressively recruited from the base of the anterior gonad that eventually develops to become the fecund tubules (Sewell et al., 1997), although Ramofafia and Byrne (2001) suggested that *H. fuscogilva* from the Solomon Islands did not conform to TRM considering the absence of distinct tubule cohorts and progressive tubule recruitment in white teatfish and the synchronous gamete development across tubule length groups. Although this may be true, the plasticity of gonad structure between geographically separated populations should be considered that may result from unique environmental conditions such as

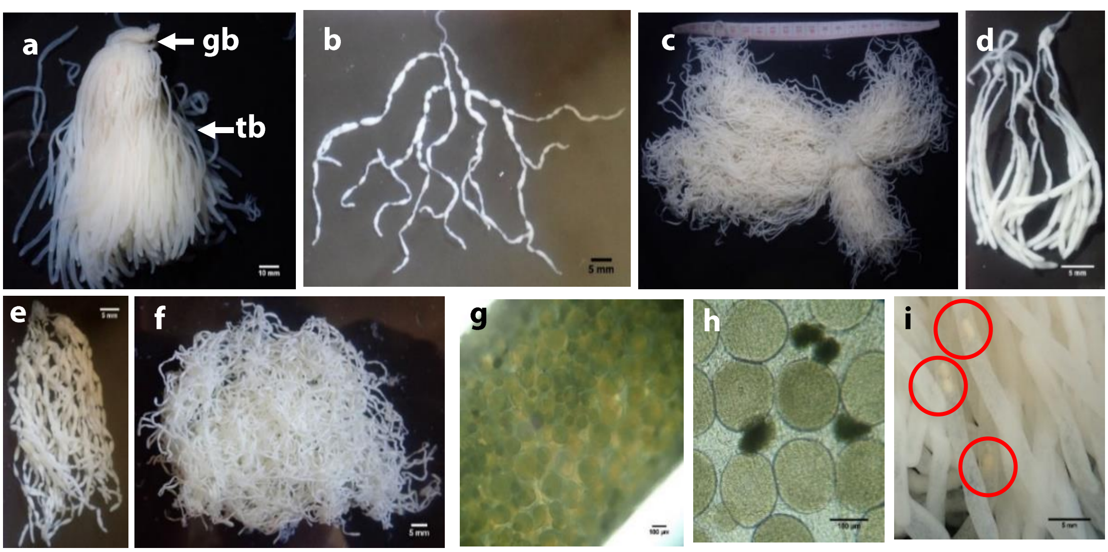

Figure 3. *Holothuria fuscogilva*. (a) Tubules (*tb*) of *Holothuria fuscogilva* originating from the gonad basis (*gb*); (b) varying frequency of bifurcation and branching in gonad tubules; (c) gonads showing the presence of tubules at different lengths; (d) growing (Stage II) male gonad tubules; (e) beaded mature male gonad tubules; (f) spent male gonad tubules with wrinkled and shrunken appearance; (g) oocytes seen through the walls of a mature female gonad tubule; (h) Phagocytes (*phg*) in partly spawned female gonads; (i) yellowish sand grain-like material inside partly spawned female gonad tubules; (j) female gonad tubule exhibiting reduced oocyte density in the lumen.

those of *Holothuria atra*, *Holothuria mexicana*, and *Cucumaria frondosa* (Sewell, 1992; Hamel and Mercier, 1996), wherein the occurrence of TRM depends on the site or location.

Table 1. Characteristics of male and female gonad tubules of *Holothuria fuscogilva* at different stages of maturity based on gonad tubule morphology showing the mean length, diameter, and the number of branches.

Maturity Stage, Sex	Length (mm)	Diameter (mm)	No. of Branches	Characteristics
Stage II - Growing				
Male	28-215	0.5-2.7	0-36	• Tubules appeared white. • Beaded appearance not distinct. • Tubules connected to the gonad basis via thin thread-like appendages.
Stage III - Mature				
Female	23-110	1.32-2.25	2-22	• Tubules become translucent. • Wall very thin with densely packed oocytes.
Male	64-125	1.09-1.49	4-22	• Beaded tubules conspicuous and the color becomes creamy-white.
Stage IV – Partly spawned				
Female	35-172	1.25-3.5	1-11	• Tubules are translucent. • Empty lumen at the distal region with yellowish grain-like structure present inside tubules.
Stage V - Spent				
Female	34-136	0.65-1.88	2-12	• Tubules are either opaque or translucent. • Tubules shrunken and wrinkled with a reduced number of oocytes in the lumen.
Male[a]	-	-	-	• Tubules are creamy-white. • Shrunken and wrinkled beaded tubules • Tubules generally empty with few sperm.

[a] Tubule measurement not available

Sex ratio

Male and female individuals were differentiated based on the macroscopic and microscopic examinations of the collected gonads, the only viable option to determine the sex of a gonochoric population with no anatomical sexual dimorphism (Slater and Chen, 2015). A total of 37 male individuals and 25 females were collected during the sampling period. Wet weights of live individuals ranged from 1,240g to 5,800g (n = 62) and did not vary significantly between males and females ($F = 0.068$; $p = 0.795$). The total sex ratio revealed that males predominated the samples at a ratio of 1.48:1 but it did not differ significantly from the 1:1 ratio ($x^2=2.32$, $p=0.05$) (Table 2).

Sex ratio of male to female white teatfish did not show significant variation which may imply that the *H. fuscogilva* population in the area is not subject to fishing pressure (Muthiga and Kawaka, 2009). This is not surprising because of the absence of active collection or trading of *H. fuscogilva* in the area. Additionally, deviations from unity in sex ratios among large aspidochirotes are rare and are only typically exhibited by species that undergo asexual reproduction (Harriot, 1982; Uthicke et al.1998; Uthicke, 1999) which is not a characteristic of *H. fuscogilva*, thus the significant deviation of sex ratio in March is still unclear. It is worth noting that reproductive success is partly dependent on the abundance and distribution of adults, and the availability of gametes of both sexes (Levitan 1991, 2005).

Table 2. Monthly sex ratio of male and female *H. fuscogilva* collected from Laguindingan, Misamis Oriental.

Month/Year	Male	Female	Ratio	x^2; $\alpha = 0.05$
February 2020	2	3	0.7:1	0.2
March 2020	12	3	4:1	5.4*
May 2020	11	8	1.4:1	0.47
June 2020	10	9	1.1:1	0.05
July 2020	2	2	1:1	0
Total	**37**	**25**	**1.48:1**	**2.32**

*significant ($x^2 > 3.841$; $df=1$; $\alpha=0.05$)

Gonad maturation

The percentage of male and female individuals at each gonad maturity stage in monthly samples is shown in Fig 4A, B). In February, all collected gonad samples were already spent (Stage V) which indicates that maturation and gamete release may have started before the sampling in February. It can be observed that the percent frequency of each gonad maturity stage fluctuates from February to July. In females, mature gonads (Stage III) have their peak in May (50%) and gradually receded in July while partly spawned (Stage IV) and spent (Stage V) gonads progressively increase in frequency. The same pattern was observed in male gonads in which the growing stage (Stage II) was abundantly present in March (83%) and deliberately decreases in May and June while mature gonads (Stage III) consistently increase in frequency from May to June with a slight decrease in July. The decrease in the percent frequency of growing and mature gonads was highlighted in the occurrence of partly spawned and spent stages. Individuals having mature (Stage III), and partly spawned (Stage IV) gonads have wet weights ranging from 1240g – 4400g and 1700g – 5800g male and female, respectively.

It can be observed that there is close synchrony in gonad maturation between sexes which agrees with the observations of Ramofafia et al. (2000) and Muthiga and Kawaka (2009) for *H. fuscogilva* in the Solomon Islands and Kenya, respectively.

Furthermore, white teatfish are known to exhibit total tubule resorption after spawning wherein individuals lack gonad tubules in certain periods of months (Ramofafia and Byrne, 2001) thus the occurrence of gonad tubules at different maturity stages indicates that *H. fuscogilva* in the sampling area were reproductively active during the sampling period. Gamete release is also evident due to the presence of partly spawned and spent gonads both in male and female *H. fuscogilva*. Mature male and female individuals were found to be larger than the previously established size at sexual maturity of about 1200g and size at first maturity of about 700g (Preston, 1993).

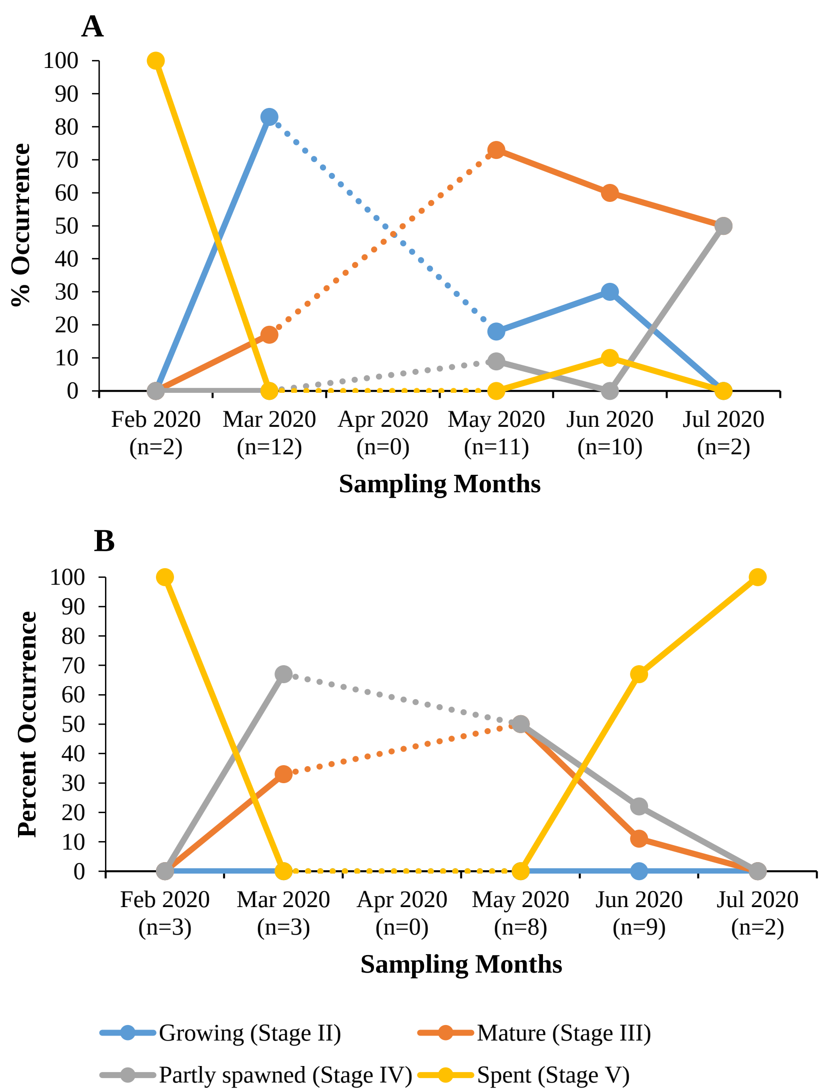

Figure 4. Percentage occurrence of gonad maturity stages of (A) male and (B) female *Holothuria fuscogilva* specimens (N=62) determined by gonad tubule morphology. (Broken lines indicates the absence of data)

Gonad index

Mean monthly gonad index (GI) for female and male *Holothuria fuscogilva* displayed synchronous (*r=0.60*; *p=0.28*) gonad development (Fig. 5). During the sampling period, the GIs for male and female *H. fuscogilva* ranged from 0.36 to 12.35 and 0.07 to 16.43, respectively which is lower than individuals in Kenya (Muthiga and Kawaka, 2009) that had a maximum gonad index of 28 but higher than those in the Solomon Islands that had maximum gonad index of 4.59 (Ramofafia et al., 2000). This observed variability in GIs among populations is evident in several studies as an implication of food availability as the driving factor that controls body size and reproductive output (Levitan, 1989; Muthiga and Jaccarini, 2005).

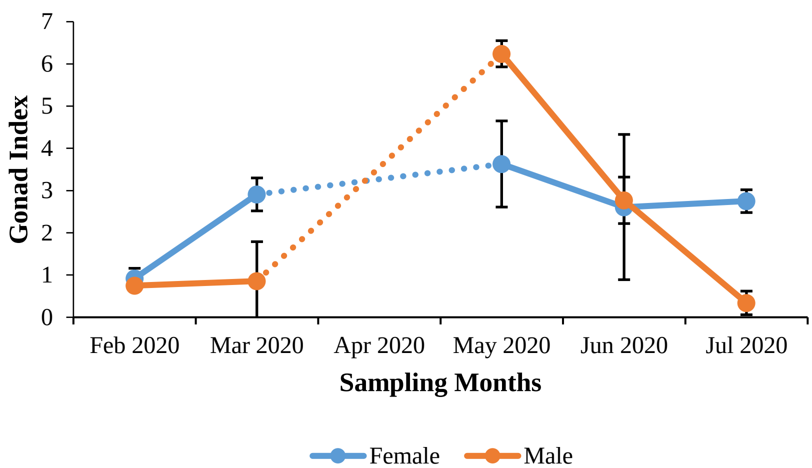

Figure 5. Monthly variation in the mean gonad index (GI) of males and females of *Holothuria fuscogilva* from Laguindingan, Misamis Oriental. (*Vertical bars* are standard errors of the mean and broken lines indicate the absence of data)

The increase and decrease of GIs for both males and females reflect the development of gonads. Ramofafia and Byrne (2001) reported an increase in GI as the size and volume of both the gonad tubule and gametes increase. Low GI in February for both males (0.92) and females (0.75) is consistent with the prevailing gonad maturity stage in February (Fig. 4). As gonad development progresses, GI gradually increases in March (male = 2.91; female = 0.88) and peaks in May (male = 3.63; female = 6.24) which may be an indication of an active vitellogenesis and spermatogenesis. On the other hand, the subsequent decline in GI and the high incidence of Stage V female gonads signify that most gamete release may have occurred in June. The evolution of the gonad index showed that *H. fuscogilva* may have actively released gametes from February to July. The spawning periods of *H. fuscogilva* were reported in the Western Central Pacific region to occur in August to October in the Solomon

Islands (Ramofafia et al., 2000), November to December in New Caledonia (Conand, 1981, 1993) December to March in the Maldives (Reichenbach, 1999) and December to April in Kenya (Muthiga and Kawaka, 2009). Spawning of *H. fuscogilva* occurred during the warm or summer season and is probably related to the annual sea temperature cycle as observed by Muthiga and Kawaka (2009). Similarly, other sea cucumber species such as *the Stichopus variegatus*, *Holothuria scabra*, and *Actinopyga mauritiana* were known to spawn during the warm season (Conand, 1993; Hopper et al., 1998; Muthiga and Kawaka, 2009) which conforms to the pattern of warm season spawning observed in most holothurians (Smiley et al., 1991). Moreover, warm-season spawning is ideal for some species because the succeeding months of low seawater temperature coupled with nutrient-rich waters are favorable for settlement and growth due to increased primary productivity (Murillo et al., 2004) ensuring higher chances of survival. Although the site of the present study is in the tropics, with most of the data collected during the warm months, we still have to collect more environmental data sets to describe the ecological parameters of the area.

This is the first attempt to study the reproductive traits of *H. fuscogilva* in the area, providing initial information on gonad morphology, gonad maturation, spawning pattern, and reproductive output. The present study was conducted in a relatively short period and that data are insufficient to establish a concrete description of the reproductive traits of *H. fuscogilva* in the area.

CONCLUSION AND RECOMMENDATIONS

The apparent absence of sea cucumber trading in Laguindingan, Misamis Oriental has possibly contributed to the balanced sex ratio of *Holothuria fuscogilva* in the area. Male and female white teatfish are sexually monomorphic and only distinct characteristics of the gonad morphology at various stages of gonad maturity delineates between sexes. Preliminary findings indicate that *H. fuscogilva* in Laguindingan, Misamis Oriental are reproductively active between February to July and that spawning may occur at these months, although more samples have yet to be collected to determine its spawning period. Size of mature male (1240g-4400g) and female (1800g-5800g) individuals were found to be larger than the previously established size at sexual maturity (1200g) and size at first maturity (700g) (Preston, 1993). The relatively high gonad indices in some samples which indicate large gonads in individuals may imply that the white teatfish population in the area has high reproductive output since larger gonads usually produce more gametes.

Although samples are reproductively active during the sampling period that coincided with the dry season (December to May), further experiments and observations should be carried out to establish the relationship. Continuous investigation and observation should be conducted that will encompass more samples, a longer period of sampling, that is at least a year and incorporating histological analysis of the gonads. Furthermore, studies on the reproductive biology and population dynamics of the species are recommended for appropriate management and conservation measures. Preliminary results from this study can serve as a reference for the LGU to implement measures to protect the white teatfish population in the area.

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