

The breeding pattern and variations in timing and reproductive output of the commercial sea cucumber *Holothuria fuscogilva* in Kenya

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Abstract—The sea cucumber *Holothuria fuscogilva* is currently one of the most commercially valuable species of sea cucumber worldwide. This study investigated reproduction of this species in order to characterize the reproductive pattern and evaluate the relationship with temperature, light and lunar periodicity. Individuals were collected monthly, between 1998 and 2007, at Shimoni, Kenya and processed using standard gonad index methods and macroscopic and microscopic observations of the gonads. Gametogenesis commenced from May and spawning occurred from December to April of each year and there was close synchrony between the sexes, contrary to the hypothesis that breeding patterns on the equator will be continuous and less synchronized between sexes. Peak spawning occurred during the last quarter of the moon. The gonad index of individuals correlated significantly with gonad tubule length and fecundity indicating that it was a good predictor of sexual maturity and reproductive output. The gonad index showed a significant correlation with temperature but not light, suggesting that temperature may play a role in controlling reproduction. The life history strategy of this sea cucumber included a higher energetic investment in the reproductive output of females and spawning at a time favorable for larval development. There was a shift in sex ratio from unity to significantly more males over the sampling period, as well as a significant reduction in mean sizes (body wall weight) and reproductive output (gonad index) suggesting that the reproductive success of this species was potentially negatively affected by fishing.

INTRODUCTION

Holothurians (sea cucumbers) have been harvested for hundreds of years in many nations of the Western Indian Ocean and an increasing demand to supply the global market, has led to unsustainable fishing and over-exploitation of natural stocks (Marshall *et al.*, 2001; Lovatelli *et al.*, 2004; Conand & Muthiga, 2007; Conand, 2008).

Several management measures including minimum size limits, gear restrictions, closed seasons and mariculture are either currently under consideration or implementation (Conand & Muthiga, 2007). These management interventions rely in part on knowledge of the reproductive biology of sea cucumbers, however, there is relatively little information on the reproductive biology

of many tropical commercial species of sea cucumbers (Bruckner, 2006; Conand & Muthiga, 2007; Conand, 2008).

The sea cucumber *Holothuria fuscogilva* is currently one of the most commercially valuable species of sea cucumber worldwide (Bruckner, 2006). It is widely distributed and harvested throughout the WIO (Marshall *et al.*, 2001; Conand & Muthiga, 2007). In previous studies on this species, an annual pattern of reproduction was reported in New Caledonia (Conand, 1993), the Maldives (Reichenbach, 1999), and the Solomon Islands (Ramofafia *et al.*, 2000) and photoperiod and temperature were reported as the controlling factors.

The Kenyan coast occurs at the equator and is exposed to monsoonal seasonality with varying oceanographic conditions of temperature, light, and productivity (McClanahan, 1988). Studies along the Kenyan coast are therefore suitable for testing the effects of monsoonal seasonality on breeding patterns of marine invertebrates. *Holothuria fuscogilva* is an important component of the catch (Muthiga & Ndirangu, 2000) and it was selected as part of a larger regional project to assess the reproduction of sea cucumber species of commercial value (Conand *et al.*, 2006). The aims of the current study were to 1) describe the reproductive pattern of *H. fuscogilva*, 2) determine the relationship between reproduction and environmental factors (temperature, light and lunar period), and 3) test the hypothesis that reproduction will show a weak seasonal pattern at the equator due to reduced variability of the environmental factors that control gametogenesis and spawning.

MATERIALS AND METHODS

The study was undertaken at Shimoni (Lat 4°S) in Kwale district, in the south coast of Kenya, between the sampling periods; December 1998 to December 1999, April 2000 to March 2001 and January 2006 to January 2007. Within each of these sampling periods, monthly samples of 10-20 *Holothuria fuscogilva* individuals were collected by fishermen using SCUBA at a depth of 18–25m. All individuals encountered

during each dive were collected irrespective of their size, placed in polythene bags (to retain any eviscerated organs) filled with seawater mixed with 0.5M MgCl₂ and transported to the Shimoni fisheries office laboratory where dissections were carried out.

Reproductive pattern

Individual sea cucumbers were dissected and processed using standard methods detailed in Muthiga & Conand (2006). Gonad indices were calculated using the formula: gonad wet weight/body wall wet weight $\times 100$. Sex and maturity stage were determined from the color and morphology of the gonad and observation of gonad tubules under a binocular microscope.

The Chi-squared test was used to test whether there was a significant difference in the number of males and females sampled (sex-ratio) in each sampling period. The relationship between individual body size and gonad growth was determined by a correlation of body wall wet weight (g) and gonad index (%). The differences between the males and females was determined through a single factor ANOVA of gonad indices and the level of sexual synchrony between the sexes was determined by a correlation between the mean monthly gonad index of females and males.

The variability in reproduction between the months and between the years was determined by testing the gonad index data for normality using the Shapiro-Wilk W test; if found not to be normally distributed, the Kruskal-Wallis test was then used to test for differences in the gonad indices between months and between years.

Reproductive output

The reproductive output of *H. fuscogilva* was investigated from measurements of 1) the gonad size (gonad wet weight (g) and gonad tubule length (mm)); and, 2) fecundity measured as the number of oocytes a female produced during the peak reproductive period (oocytes/female). Gonad tubule length and fecundity were only measured for the peak reproductive period (June – November) of the

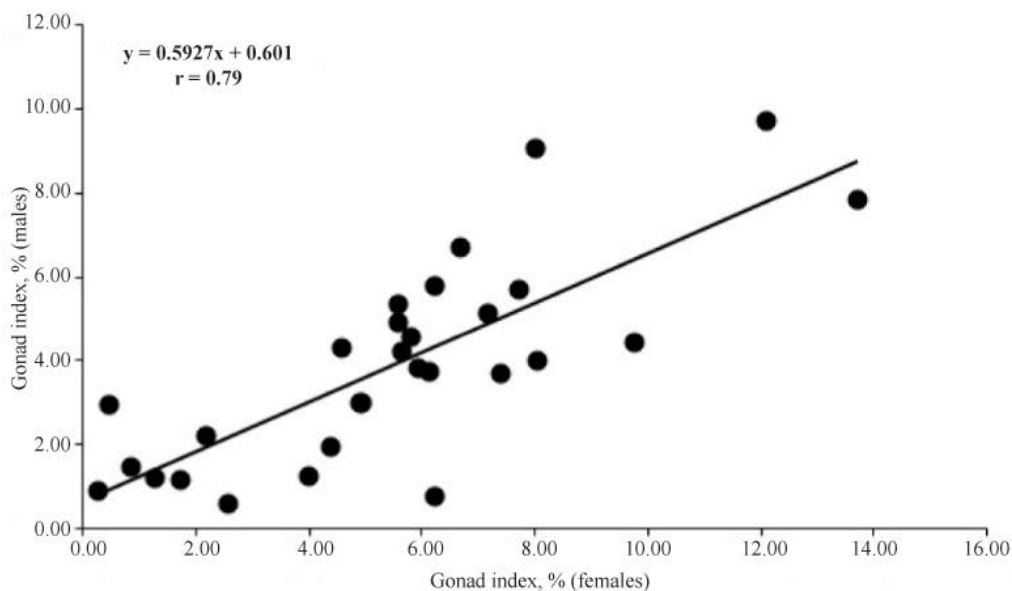


Fig. 1. The relationship between the mean monthly gonad indices of female and male *Holothuria fuscogilva* collected in Shimon, Kenya between 1998 and 2007. Ten to twenty individuals were collected monthly

2006 – 2007 samples. The gonad tubule length was measured from the gonad basis to the tip of the longest gonad tubules, while fecundity was calculated as the number of oocytes in a given weight of preserved gonad tissue using methods detailed in Muthiga (1996). The fecundity was calculated as: Oocytes /female = the average number of oocytes per aliquot $\times$ the dilution factor $\times$ the proportion of the weight of the piece of gonad to the total gonad weight. The relationship between individual and monthly averages of gonad tubules, gonad size and fecundity was determined through correlations of these parameters.

Temperature, light and lunar period

Air temperature ($^{\circ}\text{C}$) and light ($\text{Einstein}/\text{m}^2/\text{day}$) were obtained from NOAA satellite data, averaged for each month for the respective years and correlated against the mean monthly gonad index. Lunar periodicity was tested by calculating the average gonad index for each lunar day (new moon = lunar day 0) and correlating this against the lunar day.

RESULTS

Reproductive pattern

The size of the individuals collected at Shimon ranged from 115 g to 2390 g ($n = 553$) and there was no significant difference (Table 1) in the average body wall weights of males (1384 ± 19.8) and females (1330 ± 20.6). Nineteen individuals that could not be sexed had much lower body wall weights (918 ± 78). The sex ratio for samples collected in 1998 – 1999 (0.91: 1) and 2001 – 2002 (1.07:1) did not differ significantly from unity ($\chi^2 = 0.41$; $\text{df} = 196$ and $\chi^2 = 0.17$; $\text{df} = 142$ respectively). There were however, significantly more males (113) than females (82) in the 2006 – 2007 samples ($\chi^2 = 4.93$; $\text{df} = 194$; $p = 0.05$).

The relationship between the body wall weight and gonad weight of individuals was significant (Table 2) but not between weight and gonad index (Table 2). There was also a statistically significant relationship between the monthly gonad index of the sexes (Fig. 1) hence gonad index data of females and males were pooled for all years for illustration of

Table 1. The morphological characteristics of *Holothuria fuscogilva* collected in Shimoni on the south coast of Kenya. The mean ($\pm$ SEM) for females (n = 250) and for males (n = 285) and the overall means are provided. Single factor ANOVA is used for comparison between females and males

Characteristic	Females	Males	Overall	Comparison
Body weight (g)	1330.42 $\pm$ 20.64	1383.53 $\pm$ 19.80	1343.59 $\pm$ 14.48	F = 0.28; p = 0.59
Gonad weight (g)	95.41 $\pm$ 5.14	63.36 $\pm$ 3.36	75.70 $\pm$ 2.98	F = 36.55; p < 0.001
Gonad index (%)	6.19 $\pm$ 0.33	3.85 $\pm$ 0.19	4.77 $\pm$ 0.19	F = 39; p < 0.001

the annual maturity cycle (Fig. 2). The annual reproductive maturity cycle showed a seasonal pattern that varied during the different years. For example, the gonad index of individuals in July and August of 2006-2007 samples were unusually high attributable to large individuals with large gonads. Gonad indices showed the lowest values in March to April (0.1-1.4%) and increased markedly by June - July (6 - 12 %). Gonad indices remained relatively high from July to November, then decreased markedly between December and February (>4%). There was a weak but statistically significant variability in the gonad indices between the years ($\chi^2 = 7.46$; p = 0.107; df =

2) and a much stronger significant difference in the gonad indices between months ($\chi^2 = 175.1$; p = 0.0001; df = 11).

Reproductive output

Individuals had average gonad weights of 75.7 $\pm$ 2.98 g and gonad indices of 4.77 $\pm$ 0.19%. Females had on average 33% and 37% higher gonad weights and gonad indices than males (Table 1). There was also a significant correlation between the gonad weight and body wall weight of individual females (r = 0.18; p = 0.003; n = 250) and of males (r = 0.32; p = 0.001; n = 287).

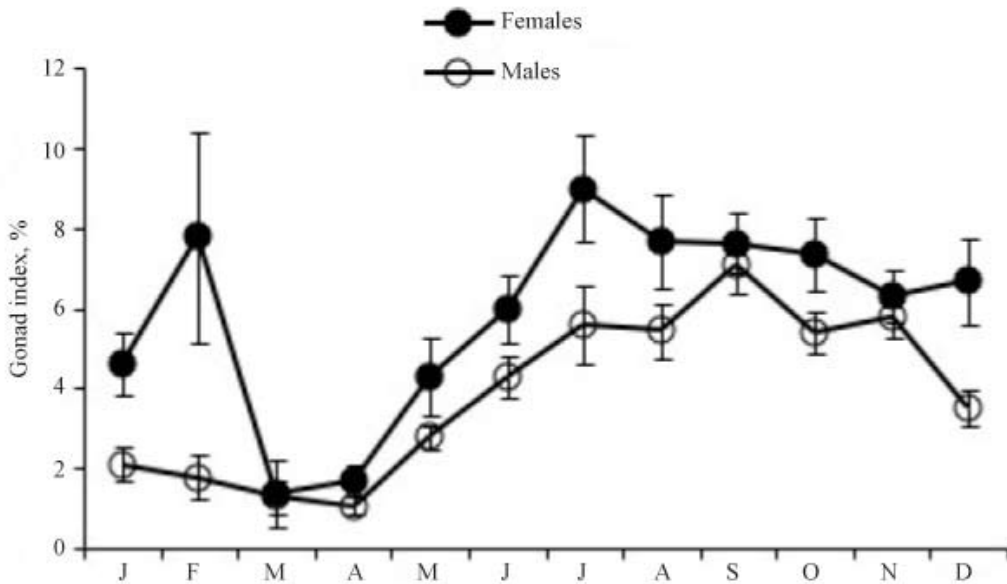


Fig. 2. The seasonal variation in the mean monthly gonad index (% $\pm$ sem) of female (closed circle) and male (open circle) *Holothuria fuscogilva* collected in Shimoni, Kenya between 1998 and 2007. Ten to twenty individuals were collected monthly

Table 2. Relationships between different morphological characteristics of *Holothuria fuscogilva* collected in Shimoni, Kenya and with environmental parameters (temperature and light). Units for gonad tubule length = mm, fecundity = $\times 10^6$ oocytes/female, temperature = °C, and light = Einstein/m²/day

Comparison	r /p value
Individual body weight vs.	
– Gonad weight (g)	r = 0.35; p = 0.05
– Gonad index (%)	r = 0.04; ns
Gonad index vs. gonad tubule length (individuals)	r = 0.47; p = 0.01
Monthly gonad index vs. monthly gonad tubule length	r = 0.51; ns
Gonad index vs. fecundity (individuals)	r = 0.54; p = 0.005
Monthly gonad index vs. monthly fecundity	r = 0.92; p = 0.0001
Gonad tubule length vs. fecundity (individuals)	r = 0.32; p = 0.05
Monthly gonad tubule length vs. monthly fecundity	r = 0.49; ns
Monthly gonad index vs.	
– Monthly light	r = 0.20; ns
– Monthly temperature	r = 0.87; p = 0.005
Mean gonad index vs. lunar day (Jul - Nov)	r = 0.41; p = 0.01

The gonad tubule lengths for individuals ranged from 5 mm to 196 mm and averaged 119.31 ± 9.97 mm ($n = 39$). The gonad tubule lengths fluctuated throughout the year but the monthly averages followed a pattern similar to the monthly changes in gonad index and were significantly correlated with the gonad index (Table 2).

Fecundity varied between 0.07 to 24.51×10^6 oocytes/female with an average of $6.45 \pm 0.88 \times 10^6$ oocytes/female and was significantly correlated with the gonad index of individual females and when averaged monthly (Table 2). Although there was a significant relationship between the fecundity and gonad tubule length of individuals, the relationship between the mean monthly fecundity and mean monthly gonad tubule length was not significant (Table 2).

Temperature, light and lunar period

The monthly air temperatures showed a seasonal pattern with a peak in March (29.1°C) decreasing to 25.0°C in July-August. Light showed a similar pattern, peaking earlier in February (53.4

Einstein/m²/day) and decreasing to the lowest levels (38.4 Einstein/m²/day) by June. There was a significant and negative relationship between the average monthly temperature and gonad index averaged over the sampling period, but not between mean monthly light and mean monthly gonad index (Table 2). The average gonad index of individuals collected on different lunar days when reproductive activity was at its highest (July - December) was variable averaging from <2% to 12% and showed a significantly negative relationship with the lunar day (Table 2).

DISCUSSION

Reproductive pattern

While seasonal reproductive patterns are typically reported for marine invertebrate species that occur in environments where seasonality is pronounced (Giese & Pearse, 1974; Smiley *et al.*, 1991), little information is available on reproduction in tropical environments that also experience seasonality but with less variation in temperature and other environmental parameters. *Holothuria fuscogilva* in this study showed a seasonal

pattern with spawning occurring between December and April towards the end of the northeast monsoon period on the Kenyan coast. The general pattern of gonad growth and spawning was repeated in the three years and although there was variability between years in the timing of the onset of gametogenesis and spawning, the differences between the months within each year was much more significant than the inter-annual differences.

The pattern of reproduction does not support the hypothesis that marine organisms at the equator will breed year round (Orton, 1920; Giese & Pearse, 1974; Smiley *et al.*, 1991). This hypothesis has also been previously challenged in studies of sea urchins and sea cucumbers that have shown annual patterns of reproduction close to the equator (Pearse, 1968; Reichenbach, 1999; Muthiga & Jaccarini, 2005; Muthiga, 2006). It has also been argued that reproductive synchrony within and between species will break down closer to the equator due to reduced variability in environmental parameters (Oliver *et al.*, 1988). Gonad growth of the sexes however, was highly correlated, indicating tight synchrony between males and females. Sexual synchrony has previously been reported in *H. fuscogilva* (Conand, 1993; Ramofafia *et al.*, 2000) and other sea cucumbers including *H. scabra* (Ramofafia *et al.*, 2003), *H. arenacava* (Muthiga, 2006) and *H. leucospilota* (Drumm & Loneragan, 2005; Gaudron *et al.*, 2008).

The gonad index of individuals did not show a correlation with body size, indicating that the gonad index method is a reliable method for tracking reproductive maturity in *H. fuscogilva* (Grant & Tyler, 1983). The gonad index increased gradually from April - May indicating the onset of gametogenesis and peaked in July - September and spawning commenced in November through to February. An annual pattern of reproduction (Table 3) has also been reported at sites closer to the equator in the Maldives (7°N; Reichenbach, 1999), the Solomon Is. (9°S; Ramofafia *et al.*, 2000), and, at higher latitudes in New Caledonia (20°S; Conand, 1993). Within this wide geographical range (+/- 16 degrees of latitude), *H. fuscogilva*

has an annual pattern of reproduction only differing in timing of the onset of gametogenesis and the length of the spawning period, which suggests that this may be the common life-history strategy for this species.

Reproductive output

The reproductive output of marine invertebrates has been shown to be dependent on several factors, including sex, the individual size and ecological factors such as population density (Levitan, 1991; Muthiga, 2006).

There was a significant relationship between the gonad weight and body wall weight of individual *H. fuscogilva* in the present study which supports findings from other studies of sea cucumbers (Conand, 1993; Muthiga, 2006). The average gonad weight and gonad indices were almost twice as high in females than in males despite females having similar body weights on average as males which is comparable to studies of *H. fuscogilva* (Conand, 1993; Ramofafia *et al.*, 2000) and other sea cucumber species (Conand, 1993; Ramofafia *et al.*, 2003; Drumm & Loneragan, 2005; Muthiga, 2006).

The average gonad indices reported for *H. fuscogilva* (mean 3.35% for males and 6.19% for females; maximum ~28%) were higher than individuals in the Solomon Is. (Ramofafia *et al.*, 2000) that had gonad indices ranging from 3.69 - 4.59% and in New Caledonia that averaged 0.8 - 2.2%. Variability in reproductive output in different populations of marine invertebrates has been reported in several studies that show food availability as the driving factor controlling body size and reproductive output (Levitan, 1989; Muthiga & Jaccarini, 2005).

The male to female ratio was at unity in 1998 - 1999 and 2000 - 2001 samples but a shift to significantly more males occurred in the 2006 - 2007 samples. A shift in sex ratio towards males has been reported in *H. scabra* populations under heavy fishing pressure in the Red Sea (Hassan, 2005) and in *H. whitmaei* in the Great Barrier Reef, Australia (Shiell &

Table 3. Summary of the reproductive periods of *Holothuria fuscogilva* across its distribution

Location	Latitude	Reproductive period	Reference
Maldives	7° N	Annual, spawning from December to March	Reichenbach, 1999
Shimoni, Kenya	4° S	Annual, gametogenesis starts from May, spawning from December to April	Present study
Solomon Islands	9° S	Annual, gametogenesis initiated in March, spawning from August - October	Ramofafia <i>et al.</i> , 2000
New Caledonia	21° S	Annual, spawning from November to January	Conand, 1993

Uthicke, 2006). Since reproductive success is partly dependent on the abundance and distribution of adults, and the availability of gametes of both sexes (Leviton, 1991; 2005), the shift to more males could significantly reduce the overall reproductive success of the southern Kenyan population of *H. fuscogilva*.

Large gonads in individuals are often interpreted as an indicator of a high reproductive output because larger gonads usually produce more gametes. There was a strong relationship between the number of gametes (measured as fecundity) and the size of the gonad (measured as gonad index and gonad tubule length) indicating that gonad size is a reliable predictor of individual reproductive output for *H. fuscogilva*. Gonad tubule length has also been shown to correlate with sexual maturity in *H. fuscogilva* in the Solomon Is. (Ramofafia *et al.*, 2002) and with gonad index in *H. whitmaei* (Shiell & Uthicke, 2006). Mature tubules ranged in size between 77 and 107 mm long, which is comparable to *H. fuscogilva* in the Solomon Is. (50 – 180 mm; Ramofafia *et al.*, 2000).

Individual *H. fuscogilva* produced up to 24 million oocytes per female with an average of 11 million oocytes per female at the peak reproductive period. This compares favorably with estimates by Conand (1993) of 6 – 14 million oocytes per female. In addition, there was a significant correlation between fecundity and female size indicating that larger females produced more eggs, which has been shown in several species of echinoderms including starfish, sea urchins and sea cucumbers (Chia, 1966; Conand, 1993; Muthiga, 1996; Toral-

Granda & Martinez, 2007). Monthly changes in fecundity correlated significantly with monthly changes in gonad index indicating that the pattern of gonad growth is a good predictor of the reproductive maturity cycle and can be used to estimate the peak time of egg availability in *H. fuscogilva* on the Kenyan coast, a parameter that is useful for mariculture. Since the highest gonad indices coincided with the highest fecundity and the longest gonad tubules, tubule length may be used as a proxy when minimum invasive procedures such as those developed by Reichenbach (1999) are required in studies of reproduction in sea cucumbers.

Temperature, light and lunar period

Temperature and light have been suggested to control gametogenesis and spawning in sea cucumbers (Smiley *et al.*, 1991; Conand, 1981; 1993; Ramofafia *et al.*, 2000; Muthiga, 2006). Both these environmental factors exhibit a seasonal pattern along the Kenyan coast (McClanahan, 1988). The gonad index of *H. fuscogilva* showed a highly significant correlation with temperature but not with light which suggests that temperature may play a role in reproduction in this species. Previous studies on the reproduction of *H. arenacava* (Muthiga, 2006) and *H. scabra* (Muthiga *et al.*, 2009) on the Kenyan coast, showed a significant correlation between the gonad index and both temperature and light. One explanation for a lack of correlation between gonad growth and light in *H. fuscogilva* tested in the present study

could be the differences in the light regime where this species occurs. Both *H. arenacava* and *H. scabra* were found in relatively shallow waters (up to 8m) while *H. fuscogilva* was collected between 18–25m. Although light was not measured directly at the study site in this study, light is possibly lower and less variable at depth than in shallow waters and will therefore not be expected to have a strong influence on the reproduction in *H. fuscogilva*.

Another aspect of light, the photoperiod (or day length), has been shown to control reproduction in starfish and sea urchins (Pearse & Eernisse, 1982; Pearse *et al.*, 1986), to influence gonad development in the sea cucumbers *H. fuscogilva* in the Solomon Islands (Ramofafia *et al.*, 2000), *Actinopyga mauritiana* in Guam (Hopper *et al.*, 1998), as well as *H. nobilis*, *Thelonata ananas*, *Stichopus variegatus* in New Caledonia (Conand, 1981; 1993) and *H. scabra* in the Solomon Islands (Ramofafia *et al.*, 2003). All these locations have larger variability in day length than more equatorial locations such as the Kenyan coast where day length varies by < 30 minutes per year. It is likely that the small change in day length on the Kenyan coast is not a sufficiently strong cue, especially in a species that occurs in deeper waters.

Moonlight has also been shown to control spawning in many species of sea cucumbers with several studies reporting spawning at the full moon (Babcock *et al.*, 1992; Mercier *et al.*, 2000; 2007). There was a correlation between gonad index and lunar day (during the peak reproductive period) in this study, with the highest gonad indices occurring during the first quarter and the lowest during the last quarter of the moon. No field observations of *H. fuscogilva* have been reported that verify lunar periodicity.

In addition to studies on echinoderms reporting annual patterns of reproduction on the Kenyan coast (Muthiga & Jaccarini, 2005; Muthiga, 2005; 2006), there is growing evidence that other marine species including finfish, barnacles and oysters (Nzioka, 1979; Ntiba & Jaccarini, 1990; Ruwa & Polk, 1994) also exhibit annual patterns of reproduction and settlement. This implies that the environmental

seasonality of the Kenyan coast probably acts as the proximate driver of the timing of gametogenesis and spawning in marine species.

CONCLUSION

The results of this study indicate that *H. fuscogilva* has an annual pattern of reproduction on the Kenyan coast, that is similar to other studies across its range of distribution, suggesting that this is the common mode of reproduction in this species. This fact and the presence of reproductive synchrony between the sexes is counter to the hypothesis that reproduction will show a weak seasonal pattern at the equator due to reduced variability of the environmental factors that control gametogenesis and spawning. Although temperature showed a significant correlation with gonad index, further experiments would have to be carried out to establish if and how temperature affects reproduction. The shift in sex ratio from unity towards significantly more males suggested a negative impact of fishing pressure, however, further studies on the effects of fishing on the population structure, growth and recruitment of this species are needed to support this finding. This is the first study of *H. fuscogilva* in East Africa and increases our knowledge of this highly valuable and widely distributed commercial sea cucumber. The study provides information including morphometric data, information on sexual maturity, reproductive seasonality, reproductive output including fecundity and gonad tubule length that are useful for mariculture and management as well as potential biological signs that reflect the impacts of overfishing.

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