

The effects of temperature and light on the gametogenesis and spawning of four sea urchin and one sea cucumber species on coral reefs in Kenya

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Abstract. Seasonality in reproduction in temperate marine invertebrates is well recognized, but reproduction at the equator where environmental variability is lower is typically assumed to be continuous. This paper reviews studies of the reproductive cycles of four species of sea urchins and one species of sea cucumber to test for seasonality and sexual synchrony along the Kenyan coast (1°S - 5°S). Gonad index measurements and macro and microscopic observations of gonads were used to evaluate monthly changes in gonad growth. The urchin *Echinometra mathaei* and sea cucumber *Holothuria arenacava* displayed a seasonal reproductive pattern with gametogenesis commencing in July during the southeast monsoons and spawning peaking between March-April during the northeast monsoons. Monthly temperature and light significantly correlated with gonad index in these species but light displayed a stronger response. The sympatric urchins *Diadema savignyi* and *D. setosum* had a monthly gametogenic cycle with *D. savignyi* spawning after full moon and *D. setosum* spawning in the last quarter. These species also showed seasonal gonad growth with *D. savignyi* following a pattern similar to *E. mathaei*. The life history strategies of these species included a high level of sexual synchrony and spawning at the time most favorable for larval growth.

Key words: Reproductive seasonality, sea cucumbers, sea urchins.

Introduction

Reproduction in echinoids and holothuroids is reported to display seasonal reproductive cycles with temperate species generally having discrete spawning periods and tropical species reproducing for longer periods throughout the year (Orton 1920, Giese and Pearse 1974, Smiley et al. 1991). Reproductive activity was therefore hypothesized to be less synchronized and continuous throughout the year at the equator since environmental variability is less pronounced (Orton 1920, Giese and Pearse 1974, Pearse 1974). However, few studies have compared the reproduction of several species of echinoderms at the same location at the equator (Pearse 1974).

Gametogenesis in echinoderms has been correlated with environmental factors such as water temperature and photoperiod, while spawning is suggested to be triggered by changes in water temperature, food availability, light intensity, water turbulence, salinity and phytoplankton blooms (Krishnaswamy and Krishnan 1967, Himmelman 1980, Engstrom 1980, Cameron and Fankboner 1986, Pearse et al. 1986, Ramofafia et al. 2000, 2003). These factors often vary spatially and temporally and

are suggested to exercise a strong selective pressure promoting reproductive synchrony and success.

The Kenyan coast occurs at the equator (1°S – 5°S) but is exposed to monsoonal seasonality with varying oceanographic conditions of temperature, light, and productivity (McClanahan 1988). This makes it suitable for testing the effects of monsoonal seasonality on breeding patterns of marine invertebrates at the equator. This paper reviews studies of four species of sea urchins and one species of sea cucumber that were carried out on the Kenyan coast (Muthiga, 2003, 2005, Muthiga and Jaccarini 2005, Muthiga 2006), to 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 studies were undertaken at Kanamai an unprotected reef and Ras Iwatine within the Mombasa marine reserve located between 3°S and 5°S. Study sites are described in Muthiga and Jaccarini (2005), and Muthiga (2005, 2006). At each site ten to twenty specimen were collected each month for a period of one to three

years between 1986 and 1998 (Table 1). The annual reproductive pattern was assessed by tracking gonad growth by measuring the monthly gonad index calculated as the monthly mean ratio of the wet weight of the gonad, relative to the wet weight of the individual for sea urchins (Gonor 1972) and of the wet weight of the body wall for sea cucumbers (Conand 1981). Macroscopic and microscopic observations were also made of the gonad to verify the sex and maturity stages.

Table 1. The locations, species, year of study and SST (mean annual) of the study sites on the Kenyan coast. The mean annual SST is calculated from NOAA data.

Reef	Lat	Species	Year	SST
Kanamai	3° 55'	<i>T. gratilla</i>	87-88	26.9
Kanamai	3° 55'	<i>D. savignyi</i>	92-93	26.9
Kanamai	3° 55'	<i>D. setosum</i>	92-93	26.9
Kanamai	3° 55'	<i>E. mathaei</i>	92-94	26.9
Ras Iwatine	4° 70'	<i>H. arenacava</i>	97-98	27.1

The relationship between individual size and gonad growth was determined by a correlation between the gonad index (%) and the weight (g) of individuals. The level of synchrony in reproduction between the sexes was determined by a correlation between the mean monthly gonad index of female and male individuals.

Temperature and light measurements were obtained from NOAA climatic data for each location and averaged monthly from 1985 to 1994 and correlated against mean monthly gonad indices for each species. The northeast monsoons start in October and end in April, while the southeast monsoons occur from May to September on the Kenyan coast (McClanahan 1988).

Lunar periodicity was measured in the sea urchins by inducing individuals to spawn using KCl every 2 to 4 days during the peak reproductive period. The gametogenic cycle was also tracked by calculating the mean gonad index every 2 – 4 days for several lunar cycles (new moon = lunar day 0) during the peak period of reproduction.

Results

There was a significant and positive relationship between individual gonad weight and body size for all species ($p < 0.0001$ for all species) and no significant relationship between gonad index and body size for any of the species (Table 2).

The pattern of gonad growth in *E. mathaei* measured through mean monthly gonad indices was seasonal and showed that gametogenesis commenced in July when gonad indices were

$3.03 \pm 0.54\%$ (sem). The gonad index then gradually increased to peak at $8.03 \pm 0.21\%$ between January and February at the peak of the northeast monsoons (Fig. 1). There was no significant difference between the overall average gonad index of females and male *E. mathaei* (Table 3.).

Table 2. The relationship between body size (weight for sea urchins and body wall weight for the sea cucumbers) and the gonad weight, and gonad index of individuals for the species sampled. For each comparison, the correlation coefficient r , and the sample size (in brackets) are provided.

Species	Size vs. gonad weight	Size vs. gonad index
<i>T. gratilla</i>	0.53 (163) *	0.08 ns
<i>D. savignyi</i>	0.60 (270) *	0.14 ns
<i>D. setosum</i>	0.56 (276) *	0.15 ns
<i>E. mathaei</i>	0.41 (100) *	0.03 ns
<i>H. arenacava</i>	0.51 (720) *	0.30 ns

* = $p < 0.0001$; the sample sizes are the same for both comparisons

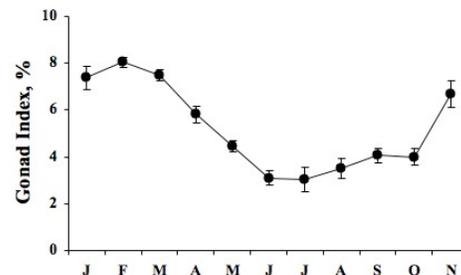


Figure 1. The seasonal variation in the gonad index of *Echinometra mathaei* (\pm sem) collected at Kanamai (3°S) between 1992 and 1994.

Monthly spawning induction showed that many individuals of both sexes were ready to spawn by October when 50% of female and 60% of male gonads had ova or sperm filling more than 50% of the gonadal lumen (Fig. 2).

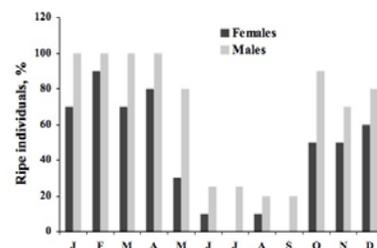


Figure 2: The percent of ripe males and females of *Echinometra mathaei* collected at Kanamai between 1992 and 1994. Ripe individuals were classified as those with gonadal lumen $> 50\%$ full of sperm or ova (Pearse 1969).

Histological observations of the gonads showed that the ovarian tubules of *E. mathaei* had in-growing nutritive phagocytes in July, new small oocytes appeared in August and ova were

present from October to May. Males were ripe for ~8 months since sperm filled more than 50% of gonadal lumen from October to May (Fig. 2), while females were ripe for a period of 5 months (December to April).

The pattern of gonad growth was also seasonal in the sea cucumber *Holothuria arenacava* (Fig. 3). Mean monthly gonad indices peaked in March when gonad indices were $13.55 \pm 0.45\%$. The main spawning commenced by August and was completed by May when gonad indices were reduced by more than 90% and all individuals had very small immature gonads.

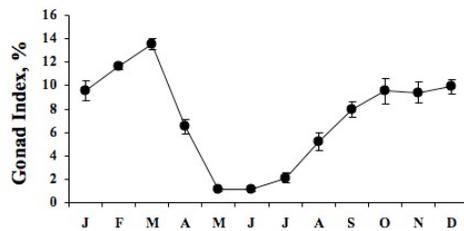


Figure 3: The seasonal variation in the gonad index of *Holothuria arenacava* (\pm sem) collected at Ras Iwatine (4° S) on the Kenyan coast.

Macroscopic examination of the gonad tubules indicated that gametogenesis commenced in July when small oocytes were present in the gonad tubules. Large oocytes were evident and filling gonad tubules by September.

The sympatric species *Diadema savignyi* and *D. setosum*, showed a monthly gametogenic cycle (Fig. 4). *Diadema savignyi* had a tightly synchronized gametogenic cycle with ~90% of individuals producing copious gametes on lunar day 18 a few days after the full moon.

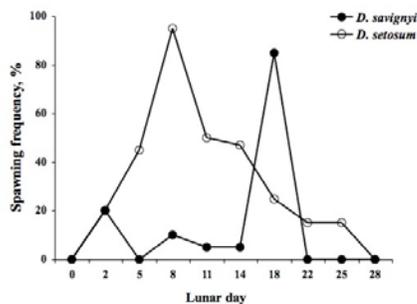


Figure 4: The spawning frequency of *Diadema savignyi* and *D. setosum* induced to spawn with injections of KCl. Spawning induction was carried out every 2 – 4 days for 3 complete lunar cycles. Lunar day 0 = new moon.

Microscopic examination of the gonads showed mature ova in gonadal lumen between lunar day 10 and 17. Spawning in *D. setosum* was less synchronized among individuals and occurred a few days after the new moon for

several days (lunar day 5 – 14) with a peak at lunar day 8. Microscopic examination showed ova in individuals from lunar day 0 to lunar day 7.

The annual pattern of gonad growth of *D. savignyi* and *D. setosum* was also seasonal but varied between these sympatric species (Fig. 5).

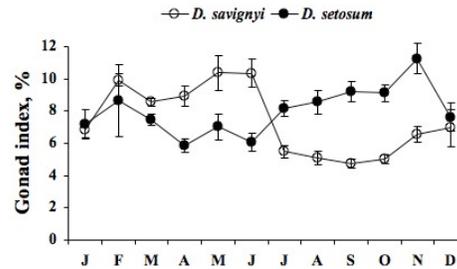


Figure 5: The seasonal variation in the gonad index of *Diadema savignyi* and *D. setosum* (\pm sem) collected at Kanamai (3° S) on the Kenyan coast.

Although mature gametes were present every month in both species, reproductive output varied and gonad indices were highest between February and June in *D. savignyi* (9.9 – 10.4%) while peak gonad indices occurred between September and November in *D. setosum* (9.2 – 11.3%).

The annual pattern of gonad growth in the sea urchin *Tripneustes gratilla* showed a weak seasonal pattern (Fig. 6) and gonad indices ranged from $2.62 \pm 0.23\%$ in January gradually increasing to $4.90 \pm 0.64\%$ in July. There was a ~30% decrease in gonad index in August.

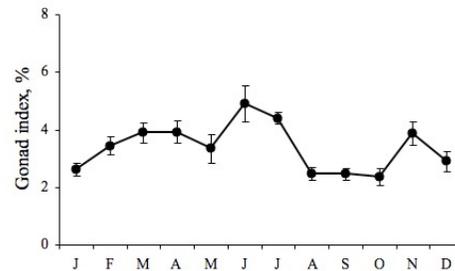


Figure 6: The seasonal variation in the gonad index of *Tripneustes gratilla* (mean monthly \pm sem) collected at Kanamai (3° S) on the Kenyan coast.

Macroscopic examination of the gonads revealed that most individuals had ‘runny’ gonads that could easily be sexed through the presence of female or male gametes. The proportion of individuals within each monthly sample with ripe gonads ranged from 0% to 75% and did not correlate significantly with mean monthly gonad index ($r = 0.33$).

The gonad indices of *T. gratilla* collected every few days over a period of two lunar cycles from April to July indicated lunar periodicity. Gonad indices increased from 3% on lunar day 0 (new moon) to 8% by lunar day 8 and lowered to < 2% between lunar days 18 to 25 during the last quarter of the moon.

The relationship between the reproductive activity of males and females measured as the average monthly gonad index in *E. mathaei*, *T. gratilla* and *H. arenacava* was highly significant but showed a lower level of synchrony in *D. savignyi* and *D. setosum* (Table 3.)

Table 3. The average gonad index of female and male individuals and the relationship between mean monthly gonad index of females and of males of the studied species. For the monthly comparison between sexes, the correlation coefficient *r* are provided.

Species	Females (GI%)	Males (GI%)	F vs. M <i>r</i>
<i>T. gratilla</i>	5.46±0.42 (55)	5.47±0.54 (47)	0.91** (12)
<i>D. savignyi</i>	8.03±0.35 (36)	7.03±0.37 (37)	0.92* (12)
<i>D. setosum</i>	9.39±0.6 (31)	8.86±0.56 (28)	0.80* (12)
<i>E. mathaei</i>	6.94±0.22 (246)	7.16±0.21 (205)	0.75** (12)
<i>H. arenacava</i>	9.8±0.3 (240)	6.8±0.4 (196)	0.79** (12)

* = *p* < 0.05 level; ** = *p* < 0.01; samples sizes in brackets

The relationship between temperature and light was variable between species. *Echinometra mathaei* and *H. arenacava* showed significant relationships with both temperature and light but the relationship with light showing the stronger response in both species (Table 4).

Table 4. The relationship between mean monthly gonad index of each species and mean monthly SST (°C), and mean monthly light (Einstein/m²/day) at the studied sites. For each comparison, the correlation coefficient *r*, and the *p* value (in brackets) are provided.

Species	Gonad index vs. temperature	Gonad index vs. Light
<i>T. gratilla</i>	-0.20 (0.52) ns	-0.43 (0.16) ns
<i>D. savignyi</i>	0.64 (0.02) ns	- 0.19 (0.55) ns
<i>D. setosum</i>	- 0.39 (0.20) ns	0.39 (0.2) ns
<i>E. mathaei</i>	0.69 (< 0.01)	0.75 (< 0.01)
<i>H. arenacava</i>	0.71 (0.01)	0.94 (< 0.0001)

There was no significant relationship between the monthly gonad index and temperature and light in *D. savignyi*, *D. setosum* and *T. gratilla*. The highest gonad indices in both *D. savignyi* and *D. setosum* however coincided with the months with the highest temperatures and light.

Discussion

The gonad index was variable across all sizes and did not show a correlation with body size in the species studied. This indicates that gonad index is independent of body size in these species and is a reliable predictor of reproductive condition as has been shown for other species of sea urchins (Gonor 1972, Muthiga 1996) and sea cucumbers (Drumm and Loneragan 2005).

The pattern of gonad growth was seasonal in all the species with *E. mathaei* and *H. arenacava* showing a particularly strong pattern that included complete spawning and resorption of gonads in May-June during the southeast monsoons followed by a period of gonad growth that peaked between February and March during the northeast monsoons.

This pattern is comparable to marine invertebrate species that occur at higher latitudes (Smiley et al. 1991, Ramofafia 2000, Drumm and Loneragan 2005) and does not support the hypothesis that marine organisms at the equator will breed year round (Orton 1920; Giese and Pearse 1974; Smiley et al. 1991). Previous studies in the tropics but at latitudes higher than the equator have also shown seasonal patterns of reproduction in sea urchins and sea cucumbers (Pearse 1969, 1974, Reichenbach 1999, Ramofafia et al. 2000, 2003).

The pattern of gonad growth in *D. savignyi*, *D. setosum* and *T. gratilla* also showed an annual pattern but in all three species, the presence of gonads and gametes in individuals during most months of the year indicated that individuals were reaching reproductive maturity and spawning at different times of the year. Gonad growth however was higher during the northeast monsoons for *D. savignyi* and *T. gratilla* and started earlier during the southeast monsoons in *D. setosum* and peaked at the beginning of the northeast monsoons.

Temperature and light, the main environmental factors that have been shown to trigger gametogenesis and spawning in marine invertebrates showed relatively small variations on the Kenyan coast. However, in at least two of the studied species, gonad growth correlated significantly with light and temperature suggesting that these factors played a role in controlling reproduction in these species.

The presence of reproductive periodicity functions to allow individuals of a population to take advantage of the best time of the year to produce young and synchronize spawning (Lessios 1981). In the studied species male and females were highly synchronized and spawning

activity generally peaked at or just prior to the time when phytoplankton concentrations peaked on the Kenyan coast.

Although the monthly ranges of temperature and light at the studied sites were low ($\pm \sim 4^{\circ}\text{C}$; ~ 20 Einstein/m²/day), the changes were predictable and followed a strong seasonal pattern that is driven by the monsoons (McClanahan 1988). This predictability could act as a reliable cue for the onset of gametogenesis and spawning.

The fact that the sympatric *Diadema* species – that also showed a monthly spawning pattern, displayed a seasonal pattern of gonad growth and the presence of seasonal reproduction in fish (Ntiba and Jaccarini 1990), in sea cucumbers (Kithakeni and Ndaro 2002, Muthiga per comm.) and recruitment of barnacles and oysters (Ruwa and Polk 1994) suggests that monsoonal seasonality maybe the proximate driver of life history strategies of marine invertebrates on the east African coast.

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