Western Indian Ocean J. Mar. Sci. Vol. 8, No. 2, pp. 193 - 202, 2009

Zooxanthellae Densities are Highest in Summer Months in Equatorial Corals in Kenya

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Keywords: Zooxanthellae, *Symbiodinium*, Mitotic Index, Coral bleaching, Kenya.

Abstract—Coral bleaching (loss of zooxathellae) is an increasing problem for the health and persistence of corals, but the phenomenon can not be fully comprehended without understanding seasonal fluctuations in the field. Seasonal dynamics of coral zooxanthellae (population density and mitotic indices) of eleven scleractinian coral species (*Acropora* sp., *Echinopora gemmacea, Favia* sp., *Galaxea fascicularis, Hydnophora microconos, Montipora aequituberculata, Pavona decussata, Pocillopora damicornis, Pocillopora eydouxi, Porites cylindrica* and *Porites lutea*) were monitored in Mombasa Marine Park from 1998 to 2006. Direct tracking of mapped corals provided evidence that zooxanthellae densities were highest during the North-East Monsoon (NEM) season and displayed highest mitotic indices during transition periods directly preceding this season. The higher densities found during the northeast monsoon (when temperatures and light radiation levels are higher) are surprising as they are contrary to trends found at higher latitudes. It is possible that at higher latitudes seasonal variability of temperatures and light is so great that it dictates zooxanthellae density dynamics, while corals closer to the Equator are less influenced and other factors may have greater influence on zooxanthellae dynamics. The present study highlights the degree of variability of zooxanthellae dynamics that may exist among coral species and compares sites from widely different geographic locations.

INTRODUCTION

Tropical reef-building corals contain micro-algae known as zooxanthellae (*Symbiodinium* spp.) within their tissue with which they exist in an obligate symbiosis that underpins the very existence of coral reef ecosystems (Muscatine and Porter, 1977). Zooxanthellae are crucial to coral polyps because they provide them with photosynthates, energy, oxygen and pigmentation (Muscatine, 1990). They in turn receive carbon dioxide, nutrients, protection and access to light (Trench, 1979). When a coral bleaches, the symbiosis is disrupted and zooxanthellae are expelled from the polyp (Hoegh-Guldberg and Smith, 1989). Severe bleaching can cause significant negative effects on coral colonies and even

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widespread mortality and degradation of coral reefs (Glynn, 1993). Mass bleaching events in the last decade have prompted increased research into zooxanthellae population dynamics, and zooxanthellae density counts are useful in quantifying bleaching responses (Fagoonee *et al.*, 1999).

The health of reef corals has been an issue worldwide for several years, and made more prominent by incidences of mass bleaching. The prevalent concept of mass bleaching is that something abnormal is occurring on corals during a time period of so called "bleaching years" that is not happening in other years. Bleaching events are often associated with extreme environmental conditions such as abnormally high sea surface temperatures and or levels of solar irradiance (Hoegh-Guldberg and Smith, 1989). In fact, it has been postulated that bleaching is the result of a seasonal cycle of zooxanthellae densities in response to seasonal environmental conditions, and that mass bleaching events are the visible result of the extreme conditions of an abnormal year (Fagoonee *et al.*, 1999). Research projects on bleaching events are recording such unusual events from field observation of discoloration of corals (Fitt *et al.*, 2000)

Several research studies from around the world have observed that zooxanthellae population densities can undergo marked seasonal fluctuations. Research in Israel (Shenkar *et al.*, 2006), Thailand (Brown *et al.*, 1999), Mauritius (Fagoonee *et al.*, 1999), the Bahamas (Fitt *et al.*, 2000; Warner *et al.*, 2002) and Hawaii (Stimson, 1997) has shown that zooxanthellae population densities are highest during colder months and lowest during warmer months, with intermediate densities in between (Table 1). These fluctuations have mostly been explained in terms of temperature and solar irradiance that affect the zooxathellae's capacity to photosynthesise (Brown *et al.*, 1999; Fitt *et al.*, 2000; Shenkar *et al.*, 2006; Stimson, 1997; Warner *et al.*, 2002). However, there are no studies documenting such trends in lower latitude areas closer to the Equator subject to less seasonal fluctuations.

The loss of symbiotic zooxanthellae involves changes in the zooxanthellae density in the tissue of reef corals. What is crucial to our understanding of zooxanthellae expulsion and bleaching is how the density of zooxanthellae within the coral is changing, if at all under prevailing environmental conditions. The marine environment in the equatorial region of eastern Africa is characterized by warm tropical conditions varying at the surface between 25°C and 31°C during the year (McClanahan, 1988; Obura 2001). There is also the east African Coastal Currents, formed by the northward deflection of the southern Equatorial Current, which flows northward through out the year, and accelerates during the southeast monsoon and slower during the northeast monsoon (McClanahan, 1998). Kenya's shallow coral reefs experienced bleaching event in 1998 during warm northeast monsoon in March and April, when local solar irradiance and water temperature were at the peak (McClanahan *et al.*, 2001). Coral reef waters are subjected to two distinct monsoon trade winds; northeast monsoon occurring from December to March while southeast monsoon occurs between May and October with 1-2 months transition periods in between that are characterized by variable and lower winds. This paper present the results of a long-term field study (1998 to 2006), with data collected on a seasonal basis, during which population density and mitotic indices of zooxanthellae for several coral species were monitored and environmental variables were measured. The late northeast monsoon and the transition period are doldrum periods when warming of surface waters is most intense between the two windy periods, and the late northeast monsoon season is when bleaching is most prevalent on the East African coast (Obura, 2005).

Table 1. A summary of zooxanthellae density fluctuations from previous studies. The study at Mombasa Marine Park is at the lowest latitude

Reference	Study location	Latitude	Study period	Species	Regular seasonal fluctuation	Lowest densities
Keshavmurthy and Fukami (2006)	Japan	≈32° N	1 year	*Acropora hyacinthus* *Stylophora pistillata*	Yes	Low SST season
Shenkar *et al.* (2006)	Israel	≈32° N	1 year	*Oculina patagonica*	Yes	High SST season
Warner *et al.* (2002)	The Bahamas	≈24° N	5 years	*Montastrea annularis* *Montastrea faveolata* *Montastrea franksi*	Yes	High SST season
Fitt *et al.* (2000)	The Bahamas	≈24° N	4 years	*Montastrea annularis* *Montastrea faveolata* *Acropora palmate* *Acropora cervicornis*	Yes	High SST season
Chen *et al.* (2005)	Taiwan	≈23° N	18 months	*Acropora palifera*	Short sampling period	
Stimson (1997)	Hawaii	≈20° N	5 years	*Pocillopora damicornis*	Yes	High SST season
Brown *et al.* (1999)	Thailand	≈7° N	4 years	*Coeloseris mayeri* *Goniastrea retiformis* *Porites lutea* *Goniastrea aspera*	Yes	High SST season
This study	Kenya	≈4° S	8 years	*Acropora* spp *Favia* spp *Pavona decussata* *Porites cylindrica* *Porites lutea* *Montipora aequituberculata* *Pocillopora damicornis* *Pocillopora eydoux* *Echinopora gemmacea* *Hydnophora microconos* *Galaxea fascicularis*	Variable	Mostly in the low SST season
Costa *et al.* (2005)	Brazil	≈7° S	1 year	*Mussismilia hartii* *Mussismilia hispida* *Siderastrea stellata*	Short sampling period	High SST season
Fagoonee *et al.* (1999)	Mauritius	≈20° S	5 years	*Acropora formosa*	Yes	High SST season

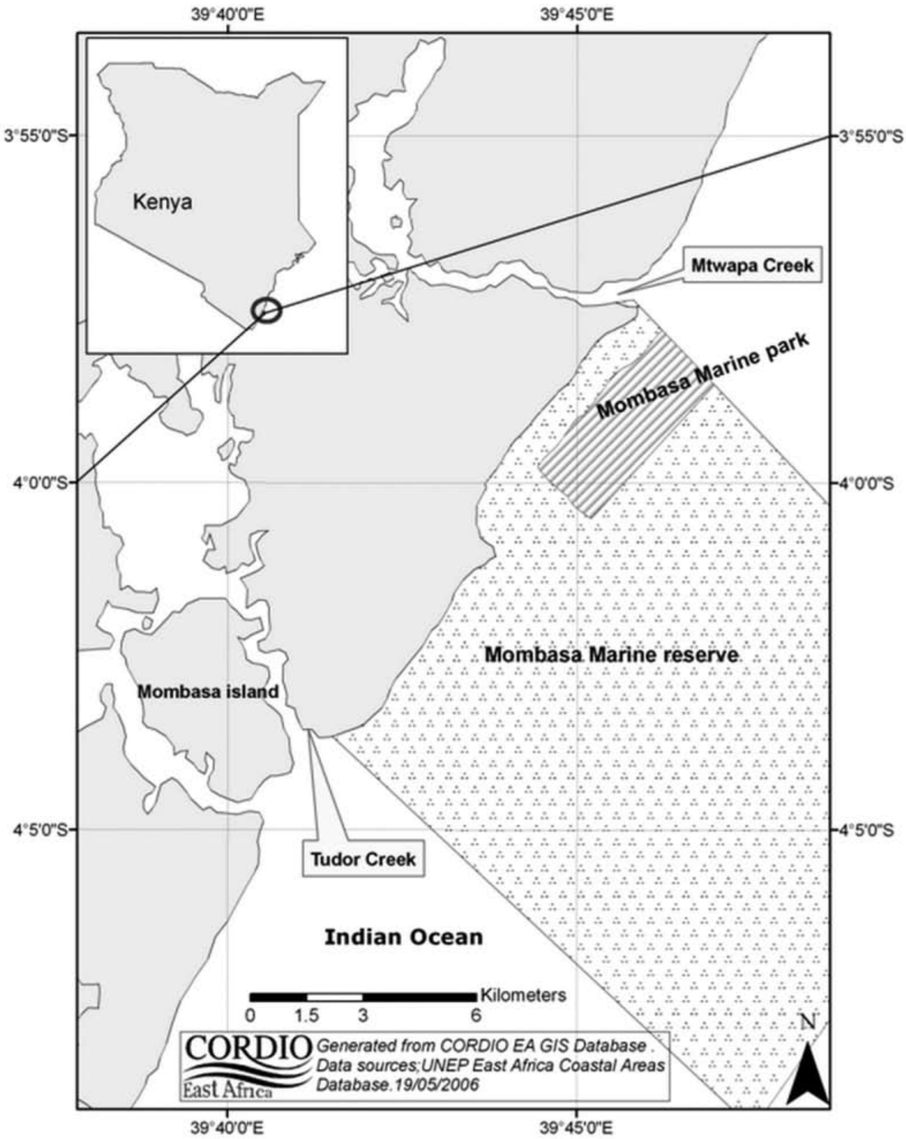

Fig. 1. Map showing location of Mombasa Marine Park

MATERIALS AND METHODS

Study site and collection of coral fragments

The study was carried out in a relatively shallow reef lagoon with a depth approximately 1-2 m at mean low tide in Mombasa Marine Park (4°S, Kenya) (Fig. 1). The eleven common species of scleractinian corals selected for this study included: *Acropora* sp., *Echinopora gemmacea, Favia* sp., *Galaxea fascicularis, Hydnopora microconos, Pavona decussata, Pocillopora damicornis, Pocillopora eydouxi, Porites cylindrica, Porites lutea*, and *Montipora aequituberculata*.

Relatively large (between 25 cm and 2 m in diameter) and healthy coral colonies for each of the target species/genera were located and mapped using labelled tags to ensure subsequent relocation and sampling from each coral colony. In case a tagged coral species got lost (i.e. due to death, very small size left) a similar coral species closer by was identified and tagged to ensure continuity of sampling replicate colonies. Periodic sampling of these coral fragments to monitor zooxanthellae density and mitotic index was done four times a year to represent four distinct seasons: the northeast monsoon (16 December-15 March), late northeast monsoon (16 March - 30 April), the southeast monsoon (1 May - 31 October), and the transition period between two main seasons (1 November - 15 December). During sampling, small coral fragments measuring about 5-10 cm in length were chopped off from the five colonies of each of eleven coral species, making a total of five samples per species, using a small chisel and hammer after which they were placed in individual pre-labelled plastic containers filled with seawater and later on taken to the laboratory in an insulated cooler. All coral fragments were regularly collected from non-shaded parts of coral colonies (margin, center), as this may affect their zooxanthellae densities (Fitt *et al.*, 2000). For branching corals, tips of branches were collected. For massive corals, it was attempted as much as possible to collect consistently from the same side of the colony.

Monitoring and determination of zooxanthellae densities and mitotic indices

In order to periodically monitor the density and mitotic indices in coral zooxanthellae, freshly collected fragments of each coral species were -washed with filtered sea water (0.45 µm mesh) using a air-brush. The samples were washed until the coral skeleton was completely white (i.e. turning white) to ensure the majority of algae (and tissue) were extracted. The resulting homogenized solution was sub-sampled twice and 1 ml of homogenate was loaded into a Sedgewick rafter chamber for zooxanthellae counts (Middlebrook *et al.*, 2008). Using a compound microscope of magnification x 400, numbers of zooxanthellae in 10 random quadrats in the Sedgewick rafter chamber were counted from replicates (n=5). To determine the mitotic index, the average number of dividing cells for two separate counts of 500 cells were made and the number of dividing cells recorded. Surface areas of the coral skeletons were determined using the ‘aluminium foil’ method (Marsh, 1970; Naumann *et al.*, 2009). In this technique, aluminium foil was wrapped over the surface of each coral piece; the foil was then removed, weighed and the surface area was estimated using a previously derived relationship between area and weight. Ambient seawater temperature (SST) data were sampled by an underwater temperature logger (Hobo Temp; Onset Corporation Ltd.), placed within a crevice of a large bommy rock (hidden to avoid being removed, stolen or damaged by tourists or fishermen) at the same site, and the daily light levels were recorded at the meteorological station operated by the Mombasa Airport.

Statistical analysis

Statistical analyses were performed using Microsoft Excel 2003 and JMP 3. Average densities and standard errors were calculated in Excel 2003. A 1-way ANOVA in JMP 3 was used to measure significant differences between zooxanthellae densities and mitotic indices for different seasons. Initially all data sets were tested for assumptions of normality and homogeneity of variance and if necessary, data were transformed to maintain normality in subsequent ANOVA.

RESULTS

Sea Surface Temperatures (SSTs) in the Mombasa Marine Park ranged from 25.5°C (August) to 29.9°C (March). Highest SSTs occur during the late northeast monsoon season while lowest SSTs occur during the southeast monsoon season (Fig. 2). Considering SSTs, the maximum temperature difference between

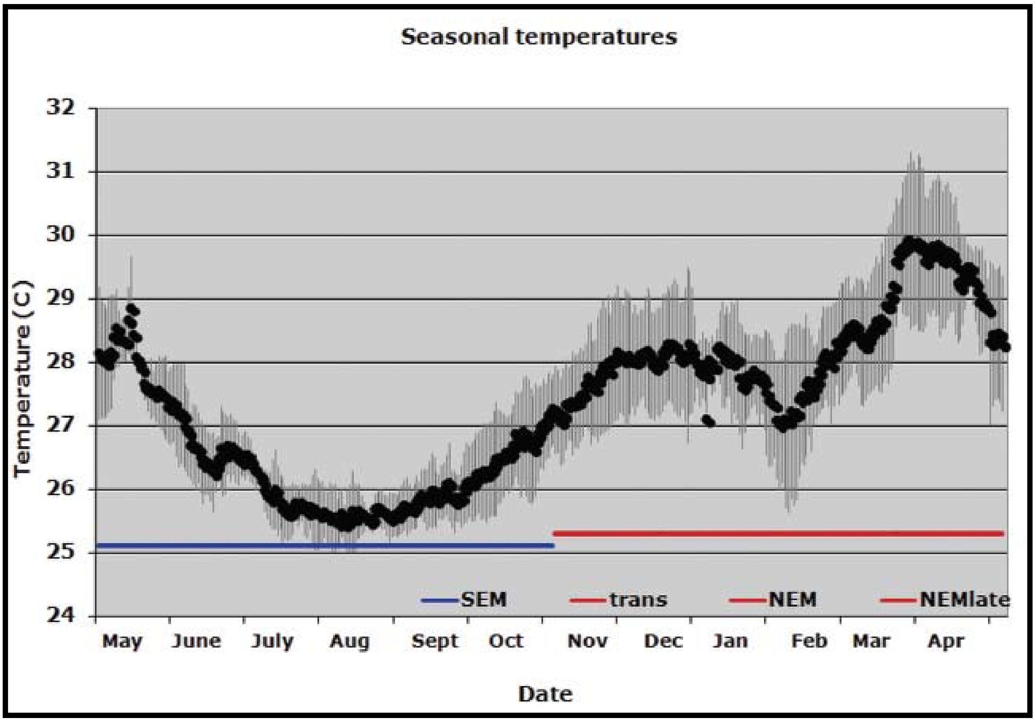

Fig. 2. Temperature data for the Mombasa Marine Park showing daily means and standard deviation for 1999-2005. Horizontal bars show seasons described in the text. NEM = Northeast monsoon, Late NEM = Late Northeast monsoon, SEM = Southeast monsoon, Trans = Transition period

southeast monsoon and northeast monsoon is almost 5°C. Photosynthetically-Active Radiation (PAR) levels in Mombasa range from 17.2 mj m^{-2} (June) to 23.2 mj m^{-2} (February). Highest light radiation levels occur during the northeast monsoon season while lowest light radiation levels occur during the southeast monsoon season (Fig. 3). Temperatures (Fig. 2) and light radiation levels (Fig. 3) are lowest during the southeast monsoon season and highest during the late northeast monsoon season. Zooxanthellae densities in relation to seasons ranged from 1.14 million cells cm^{-2} to 4.06 million cells cm^{-2}.

Although different zooxanthellae densities in different species peaked in different months, all species sampled displayed highest densities at some point during the overall northeast monsoon season (1 November to 30 April) and most displayed highest mitotic indices during the transition period directly preceding the northeast monsoon season (1 November to 15 December) (Fig. 4). One group of species (*Acropora, Porites cylindrica, Galaxea fascicularis* and *Pavona decussata*) displayed significantly higher zooxanthellae densities during the Northeast monsoon and significantly higher mitotic indices during the preceding transitional period($p<0.05$ and $p< 0.05$ respectively). A second group of species (*Porites lutea, Pocillopora damicornis* and *Echinopora gemmacea*) displayed significantly higher zooxanthellae densities during the transitional period preceding the Northeast monsoon and significantly higher mitotic indices during the same transitional period($p<0.01$ and $p<0.01$ respectively). A third group of species (*Favia* and *Montipora aequituberculata*) displayed significantly higher zooxanthellae densities during the late Northeast monsoon and significantly higher mitotic indices during the preceding Northeast monsoon ($p<0.001$ and $p<0.01$ respectively). The fourth group

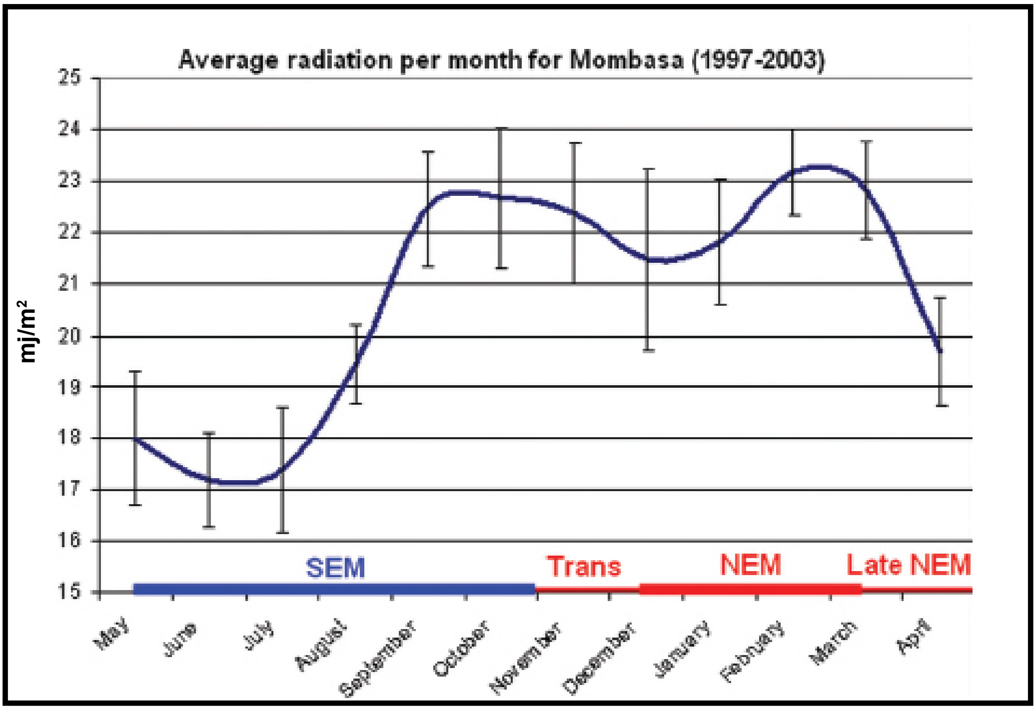

Fig. 3. Radiation data for Mombasa showing monthly means and standard deviation for 1997-2003. Horizontal bars show seasons described in the text. NEM = Northeast monsoon, Late NEM = Late Northeast monsoon, SEM = Southeast monsoon, Trans = Transition period. Data courtesy of Mombasa Meteorological Office

of species (*Hydnophora microconos* and *Pocillopora eydouxi*) displayed no significant difference between zooxanthellae densities in different seasons($p>0.05$), but did display peaks in mitotic index during the transitional periods.(Fig. 4).

A trend in the data shows peaks in mitotic index occurring directly before or during the season of highest zooxanthellae density. In all species except for *Favia* spp. and Montipora aequituberculata there is are peaks in mitotic index during the transition period going into the northeast monsoon season as SSTs and light radiation levels increase (Fig. 4). This coincides with or is followed by a peak in zooxanthellae density occurring either during the transition period or the northeast monsoon season. There thus seems to be an increased rate of zooxanthellae reproductive activity followed by a logical peak in zooxanthellae abundance.

DISCUSSION

This study shows that all species (except for *Hydnophora microconos* and *Pocillopora eydouxi* which display no significant seasonal variation) display highest zooxanthellae densities and mitotic indices at some point during the transition period, the northeast monsoon season or the late northeast monsoon season when sea water temperatures and radiation levels are highest. This shows evidence of some regulatory mechanism, as zooxanthellae density from one season to the next is dependent on the density during the preceding period. Higher temperatures and increased radiation are known to cause coral bleaching (decreased levels of zooxanthellae) during northeast monsoon through photo inhibition resulting to decreased rates of zooxanthellae (Fittal *et*

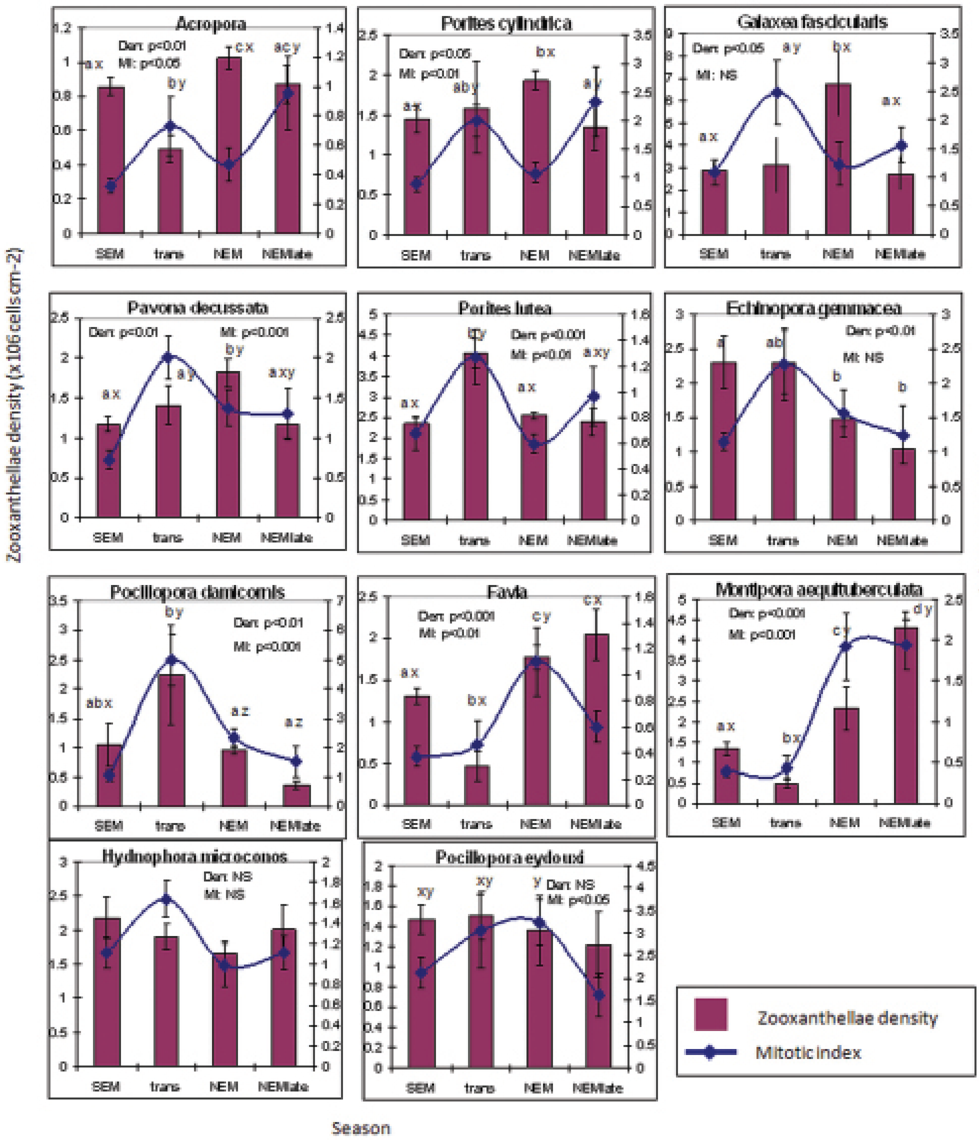

Fig. 4. Average seasonal zooxanthellae densities and mitotic indices for all species. SEM = Southeast monsoon. Trans = Transition period between monsoons. NEM = Northeast monsoon. NEMlate = Late Northeast monsoon. ANOVA values are presented, and Fisher's LSD post-hoc analyses are shown to indicate significant differences between seasons. For seasonal differences in densities, post-hoc analyses are indicated by letters a, b, c and d, while for mitotic indices post-hoc analyses are indicated by letters x, y and z. Den = Zooxanthellae density. MI = Mitotic index

al., 2000, Hoegh-Guldberg and Jones 1999). The low zooxanthellae densities found during the colder southeast monsoon season and the high densities found during the late northeast monsoon season for some species are surprising, as they are contrary to trends found at higher latitudes (Fagoone *et al.*, 1999). The 5°C difference is markedly lower than differences of 13°C in higher latitude areas (Fitt *et al.*, 2000; Warner *et al.*, 2002). It is possible that at higher latitudes seasonal variability of temperature and light is so great that it dictates zooxanthellae density fluctuations, while corals closer to equator may be less influenced by seasonal variability of temperature and light, and other factors may have greater influence on zooxathellae fluctuations to a greater extent. Nearshore reefs can be subjected to varying salinity levels driven by rainfall during the monsoon winds and influence the coral zooxanthelae densities (Obura, 2001; Fagoonee *et al.*, 1999). For example, it is possible that lower water salinities during periods of higher rainfall (for example the southeast monsoon season) could also effect the coral physiology and hence zooxanthellae densities of coral species. It is possible that at this low-latitude site, salinity levels or other locally-fluctuating environmental conditions could override the effects of sea water temperature and light radiation on coral zooxanthellae dynamics. Further research including salinity measurements in this shallow reef is necessary to provide a better understanding of zooxanthellae density variability.

CONCLUSION

In conclusion, this study shows a clear trend in increased mitotic indices during the seasonal transition period, followed directly by increases in zooxanthellae densities during the northeast monsoon season when SSTs are high. This trend is contrary to long-term analyses conducted on more temperate corals. This study thus highlights the degree of variability in zooxanthellae population dynamics there may exist among coral species and between sites at widely different geographic locations.

Acknowledgements–We would like to thank the Swedish International Development Agency, the IUCN Climate Change and Coral Reefs Working Group, the Kenya Wildlife Service and the Kenya Marine and Fisheries Research Institute for their continued support in making this study possible.

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