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Population dynamics of zooxanthellae during a bacterial bleaching event

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Abstract Each summer 80–90% of the colonies of *Oculina patagonica* undergo bleaching off the Mediterranean coast of Israel. To investigate fluctuations through a yearly bleaching cycle, monthly measurements of zooxanthella density, mitotic index and chlorophyll-*a* concentration were conducted. Results showed (1) a significant negative correlation between sea surface temperature (SST) and zooxanthella density; (2) both significantly lower zooxanthella mitotic index and higher chlorophyll-*a* per zooxanthella content during the bleaching season compared with the non-bleaching period; (3) prior to bleaching, a lag between the peak of zooxanthella density and chlorophyll-*a* concentration followed by a similar lag during recovery. Zooxanthella density declined significantly between March and May while chlorophyll-*a* concentration peaked in April, and then declined. Zooxanthella density increased significantly in November while chlorophyll-*a* concentration increased significantly in January. We conclude that during bacterial bleaching events, zooxanthellae are severely damaged. However, by the time of the following bleaching event the coral tissues regain their “normal” (pre-bleaching) zooxanthella population density.

Keywords Coral bleaching · Mediterranean Sea · Zooxanthellae · Chl *a* · *Oculina patagonica* · *Vibrio shiloi*

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Introduction

Coral bleaching is generally characterized by expulsion of the endosymbiotic dinoflagellates (zooxanthellae), loss of algal pigmentation or both (Hoegh-Guldberg and Smith 1989; Glynn 1993; Brown 1997). This phenomenon threatens the health and persistence of some of the most diverse and economically important marine ecosystems on earth (Hoegh-Guldberg 1999). Higher than average sea surface temperatures (SST) have been suggested as the primary cause of mass bleaching events (Hoegh-Guldberg and Smith 1989; Glynn and DeCroz 1990; Hoegh-Guldberg 1999; Jokiel 2004). However, in the case of the encrusting coral *Oculina patagonica*, the causative agent for bleaching is the bacterium *Vibrio shiloi* (Kushmaro et al. 1996, 1997) and seawater temperature is a contributing factor (Kushmaro et al. 1996; Rosenberg and Ben-Haim 2002). Every summer since 1993, 80–90% of the colonies of *O. patagonica* undergo bleaching in the Mediterranean Sea, off the coast of Israel (Fine et al. 2001; Israely et al. 2001). This occurs when the SST rises above 26°C, to a maximum of 30–31°C. Sea water temperature drops during the winter, and most of the *O. patagonica* colonies recover by the following spring (Fine et al. 2001; Shenkar et al. 2005). The aim of the present study was to investigate possible fluctuations in zooxanthella population dynamics and chlorophyll-*a* concentration during and following the bleaching cycle of *O. patagonica*. Such measurements may furnish a better understanding of the relative contribution of zooxanthella density versus chlorophyll-*a* concentration to the bleaching phenomenon of *O. patagonica*.

Materials and methods

The study was conducted during 2002–2003 at Hadera (Mediterranean coast of Israel: 32°29.77'N, 34°53.23'E). Surface seawater temperatures (SST) were obtained using an Onset Stow Away data-logger placed at the

study site at a depth of 1.5 m. Zooxanthella density, mitotic index (Wilkerson et al. 1983) and chlorophyll-*a* concentration were measured each month from February 2002 to February 2003. Equal-sized core samples (1 cm diameter and 2–3 mm thick) were sampled in December 2001 from three large *O. patagonica* colonies, and each attached with liquid epoxy glue to a PVC plate (7×7 cm) and returned to the sea in order to allow acclimatization and growth (Fig. 1). Each month from February 2002, 7–9 PVC plates were collected for analysis, 2–3 plates from each source colony. Coral tissue was removed from the skeleton using a jet of recirculated 0.45 µm filtered seawater (FSW) using a WaterPik® (Teledyne, USA, Johannes and Wiebe 1970) and centrifuged at 4,500g for 20 min in 50 ml tubes. In order to separate zooxanthellae from the host tissue, the liquid extract was discarded and the pellet was re-suspended in 1 ml FSW, homogenized and transferred to 1.5 ml Eppendorf tubes. Following centrifugation at 20,000g for 10 min, the liquid extract was discarded and the pellet was re-suspended in 1 ml FSW. This step was repeated twice in order to obtain a clean sample.

Density of zooxanthellae in each sample was determined from counts of three sub-samples (10 µl each), which were viewed using an improved Neubauer Haemocytometer (Weber, England). Counts were normalized to coral surface area, measured with a caliper to the nearest millimeter. The mitotic index was calculated as the percentage of doublet cells out of the total cell count in the sample (Wilkerson et al. 1983). To ascertain if the changes occurring in the coral-algal association were due to the overall numbers of zooxanthellae and/or reduction in overall chlorophyll-*a* concentrations or chlorophyll-*a* concentration/algal cell, the pellet containing the zooxanthellae was further assayed by immersion in 1 ml chilled 90% acetone overnight. The extracted chlorophyll was quantified spectrophotometrically and chlorophyll-*a* concentration was calculated

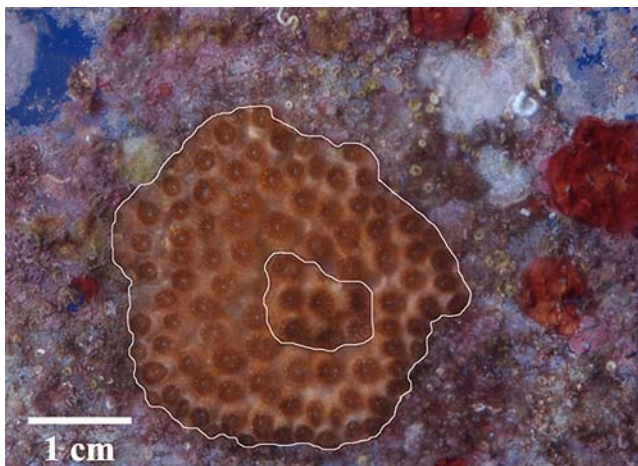


Fig. 1 *Oculina patagonica*: a PVC plate with a coral fragment. The inner line denotes the initial coral size in December 2001 and the outer line demarcates growth to August 2002

using the equations of Jeffrey and Humphrey (1975) and normalized to coral surface area.

All statistical analyses were carried out using Statistica 6.1. The data were tested for normality and homogeneity of variances. Statistics of mitotic index were carried out after arcsin transformations. First, in order to determine whether there is an influence of the colony genotype on the measurable factors throughout the year, a nested design ANOVA was carried out for each of the dependent factors (zooxanthella density, chlorophyll-*a* concentration, mitotic index transformed data and chlorophyll-*a* per zooxanthella) taking into account the fragment source. Since this test proved that none of the factors was significantly ($p > 0.05$) influenced by the source colony, the monthly samples were treated as replicates. An ANOVA was carried out in order to ascertain if there are significant differences between the measured factors throughout the year. Fisher's least significant differences (LSD) tests were used as post hoc comparisons when significant differences were detected. Linear regression analysis was used to determine whether the zooxanthella density and chlorophyll-*a* concentrations were associated with SST. Results are presented as averages \pm standard errors throughout the text.

Results and discussion

The current study is among the few field studies that have followed the monthly dynamics of zooxanthella populations during bleaching and recovery (Fitt et al. 1993; Jones 1997; Lasker 2003), and is the first study to monitor zooxanthella fluctuations during a bacterial bleaching event. Although the research was not conducted in a tropical coral reef ecosystem, it nonetheless provides important information regarding the bacterial bleaching phenomenon and coral bleaching in general.

The results show a significant association between water temperature and zooxanthella density ($r^2 = 0.68$, $y = -0.413x + 12.513$, $p < 0.01$, Fig. 2). Zooxanthella densities were significantly lower during the bleaching period (June–December, $1.1 \pm 0.1 \times 10^6$ cells cm^{-2} , $n = 53$) than during the recovery period (February–May, $5.8 \pm 0.5 \times 10^6$ cells cm^{-2} , $n = 36$, one-way ANOVA followed by Fisher LSD, $p < 0.01$, Fig. 3a). During the summer, zooxanthella density declined by approximately 95% when compared to the winter peak ($8.8 \pm 1.8 \times 10^6$ cells cm^{-2} in March, $n = 9$) and reached a minimum of $0.46 \pm 0.1 \times 10^6$ cells cm^{-2} in September, $n = 9$ (Fig. 3a). This reduction in zooxanthellae density is relatively high when compared with similar reports from corals from tropical reef systems where there was a 72% decrease in zooxanthella density in *Montastraea franksi* (Edmunds et al. 2003) from the Florida Keys, a 75% decrease in *Montastraea annularis* (Porter et al. 1989) in the Caribbean and a 66% zooxanthella decrease in *Acropora formosa* in the Great Barrier Reef (Jones and Yellowlees 1997). This difference may be a result of

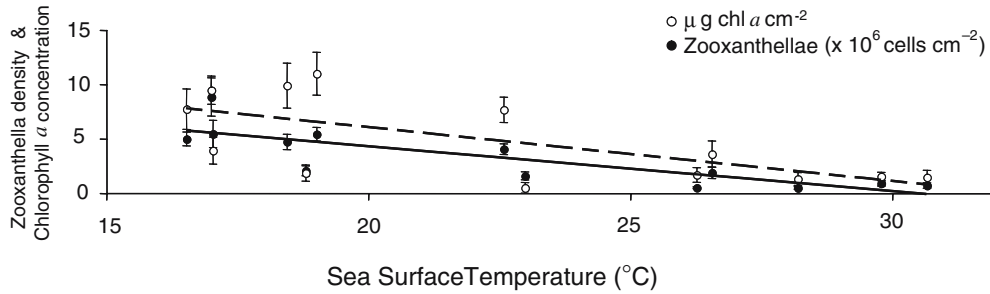
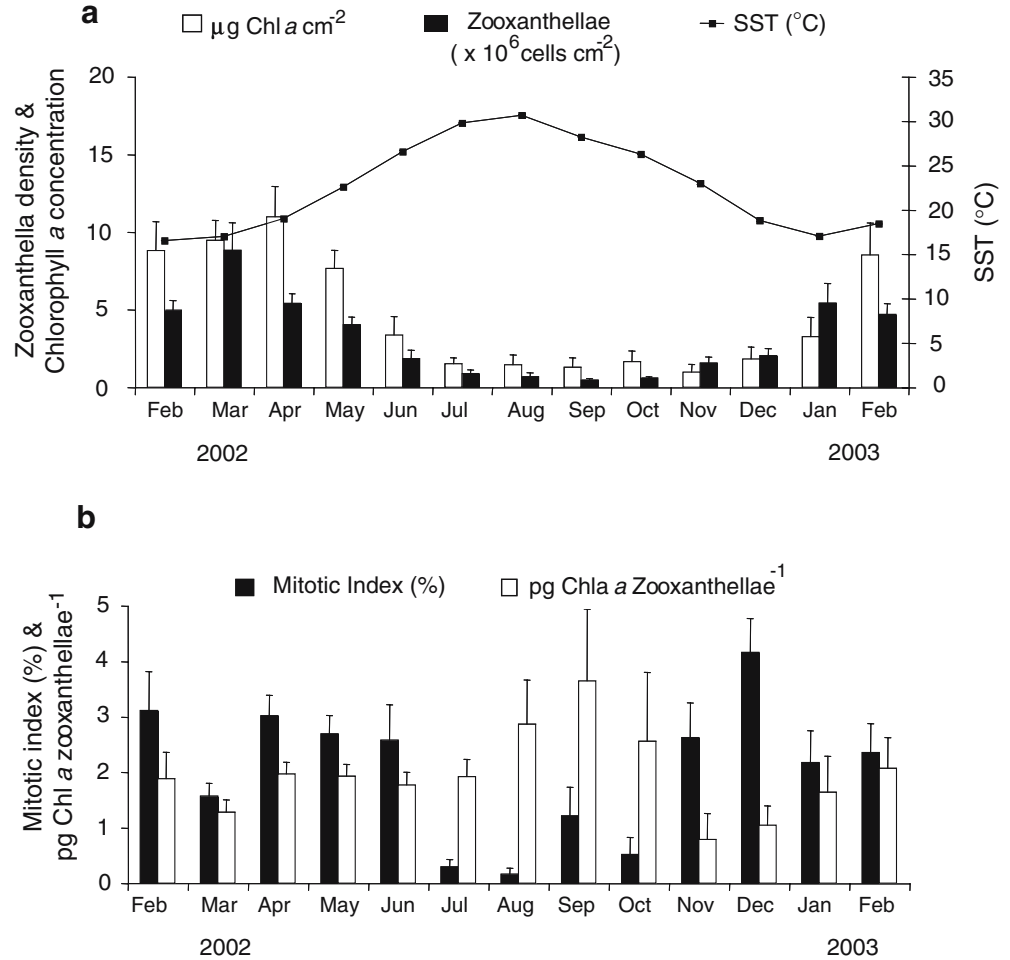


Fig. 2 *Oculina patagonica*: The relationship between mean density (\pm SE) of zooxanthellae (black circles) and chlorophyll-*a* concentration (blank circles) to SST (sea surface temperature) during February 2002 to February 2003 ($n = 105$)

Fig. 3 *Oculina patagonica*:
a Mean density (\pm SE) of zooxanthellae (black bars) and chlorophyll-*a* concentration (blank bars) during February 2002 to February 2003 ($n = 9$, SST: sea surface temperature).
b Average (\pm SE) mitotic index (black bars) and chlorophyll-*a* content per zooxanthellae (blank bars) during February 2002 to February 2003



different levels of stress experienced in each of these cases, e.g., combinations of light and temperature or other factors. It may also be explained by the comparatively high initial algal densities observed in *O. patagonica* during the winter, which may be a result of inhabiting waters with high nutrient levels due to anthropogenic activity (Ben-Yami 1998; Brown et al. 1999).

Similar to algal density, the chlorophyll-*a* concentrations were negatively associated with water temperature ($r^2 = 0.45$, $y = -0.4952x + 15.912$, $p < 0.01$, Fig. 2) with a 92% decline in the total amount of chlorophyll

($10.99 \pm 2.0 \mu\text{g chl } a \text{ cm}^{-2}$ in April, $n = 9$ compared to $0.98 \pm 0.5 \mu\text{g chl } a \text{ cm}^{-2}$ in November, $n = 7$). Interestingly, the change in chlorophyll-*a* concentration was not concurrent with the change in zooxanthella density. Between March and May there was a significant (55%) decline in zooxanthella density (one-way ANOVA followed by Fisher LSD, $p < 0.05$) while the chlorophyll-*a* concentration peaked a month later, in April, and was significantly lower in June. This lag was also observed throughout the recovery period, with zooxanthella density increasing significantly from September to November while the chlorophyll concentration increased

significantly only in January. The increase in zooxanthella density in November correlates with the steep rise in mitotic index observed in this period (Fig. 3b). Nakamura et al. (2003) reported an opposite trend showing that following bleaching in *Stylophora pistillata*, recovery occurred first due to an increase in chlorophyll-*a* followed by an increase in the zooxanthella density. This disparity may be due to a variety of causes, e.g., different morphological characters of the two corals (branching vs. encrusting), species differences, or due to different zooxanthellae clades and others. Unfortunately, at this stage, the cause of the difference remains to be shown.

Zooxanthella densities declined between March–May. In contrast, visually apparent bleaching, observed from photographs only, started to be seen in June (Shenkar et al. 2005). This shift indicates that similar to reef corals (Jones 1997), by the time bleaching was noticeable in the field, a decline of approximately 80% in zooxanthella density had already occurred ($8.8 \pm 1.8 \times 10^6$ cells cm^{-2} , $n=9$ in March, compared to $1.85 \pm 0.5 \times 10^6$ cells cm^{-2} , $n=8$ in June).

Many studies have reported an inverse relationship between zooxanthella density and mitotic index in corals following bleaching events (Hoegh-Guldberg and Smith 1989; Fitt et al. 1993; Jones and Yellowlees 1997). This relationship is often explained by the fact that the remaining zooxanthellae are situated in a nutrient-rich intracellular environment and, hence, proliferate faster than zooxanthellae in corals with higher densities of zooxanthellae (Wilkerson et al. 1983; Fitt et al. 1993). The current study presents an opposite trend. During the bleaching season (July–October) the mitotic index in *O. patagonica* was significantly lower (one-way ANOVA followed by Fisher LSD, transformed data, $p < 0.01$), only $0.6 \pm 0.2\%$ ($n=31$) in comparison with $2.77 \pm 0.2\%$ during the remaining months ($n=68$, Fig. 3b). Cervino et al. (2004) describe a similar outcome in corals affected by Yellow-Band disease. Compared with healthy tissue, diseased coral samples had a 50% decrease in algal density and an 80% decrease in mitotic index. One explanation for this reduction in mitotic index may be that *V. shiloi*, the causative agent for bleaching in *O. patagonica*, may negatively affect cell division perhaps by producing extracellular toxins (Ben-Haim et al. 1999). The high chlorophyll-*a* per cell content found during bleaching events may be explained by reduced competition for nutrients with other zooxanthellae (Fitt et al. 1993; Le Tissier and Brown 1996; Jones and Yellowlees 1997; Brown et al. 1999; Edmunds et al. 2003). In the current study chlorophyll-*a* per cell was significantly higher (*t*-test, $p < 0.01$) in July–October (2.8 ± 0.5 pg zooxanthellae $^{-1}$, $n=31$) in comparison to the rest of the year (1.6 ± 0.1 pg zooxanthellae $^{-1}$, $n=71$, Fig. 3b). However, this rise might be an artifact since the absorption peak (664 nm) used to compute chlorophyll-*a* concentrations is also affected by breakdown products and other pigments (Le Tissier and Brown 1996) remaining in the coral host. Since in *O. patagonica*

bacterial infection results in zooxanthella degradation (Ben-Haim et al. 1999), this latter explanation is most likely.

The significant decline found in the current study in terms of zooxanthella density, chlorophyll-*a* concentration and mitotic index, all indicate that during bacterial bleaching events, zooxanthellae are severely damaged. However, as the SST starts to decline the coral tissues recover and regain their normal zooxanthella population density by the time of the next bleaching event. The mechanism of this phenomenon and physiological effects of the bacteria on the zooxanthella population remain to be studied. The disparity between bleaching and recovery mechanisms in bacteria and non bacteria-induced bleaching may act as a possible diagnostic tool for studying coral bleaching.

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