

Original paper

Spatial and temporal dynamics of symbiotic dinoflagellates (*Symbiodinium*: Dinophyta) in the perforate coral *Montipora capitata*

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Abstract The illuminated parts of many hermatypic corals have higher levels of photosynthetic pigments and symbiotic dinoflagellates belonging to the genus *Symbiodinium* than the shaded parts that often appear pale. In the field, we observed that the perforate coral *Montipora capitata* restored this original pattern after being overturned by disturbance. Here, two hypotheses were tested to account for this phenomenon, using experimentally overturned *M. capitata* plates in the laboratory. Our results indicated that restoration of the original pattern was not due to changes in chlorophyll *a* content per *Symbiodinium* cell or alterations in symbiont density through differential division rates. Instead, *Symbiodinium* cells appear to be translocated within the colony. This finding demonstrates an additional photoacclimatory process for hermatypic corals, whereby new *Symbiodinium* cells proliferate in shaded parts of the colony where they are protected from potentially high levels of solar irradiance and are then transported to illuminated parts of the colony (and possibly released from the colony when damaged). Additional studies in other species are required to determine if this dynamic spatial and temporal process is a general prop-

erty of the hermatypic coral-*Symbiodinium* relationship.

Keywords coral reef, scleractinian, symbiosis

Introduction

Coral reef ecosystems are characterized by high biodiversity, gross productivity and biostructural complexity across many scales. Hermatypic (reef-building) corals are one of the cornerstone species, both because they form the carbonate structures that make up the reef itself, as well as being significant primary producers in the food web. All hermatypic corals form mutualistic symbioses with photosynthetic dinoflagellates belonging to the genus *Symbiodinium* Freudenthal (Taylor 1973). This relation dates back to the mid-Triassic (Stanley and Swart 1995), and is considered a major factor in the success of coral reefs in tropical, oligotrophic seas (Muscatine and Porter 1977).

Because of the importance of *Symbiodinium* photo-

synthesis to their hosts, much work has been done on photoacclimation in these symbioses. Two modes have been documented: 1) changes in the symbiont cells themselves, such as altering the concentration of photosynthetic pigment, change in photosystem size, and/or in enzymes of the electron transport chain (Chang et al. 1983; Masuda et al. 1993) and 2) changes in symbiont density within the host tissues (Kinzie et al. 1984; Kaiser et al. 1993; Yamashita et al. 2009). In the latter case, *Symbiodinium* densities appear to be well regulated in coral tissues, with $\sim 10^5$ – 10^6 algal cells per square centimeter of host surface area (Jones and Yellowlees 1997). Several processes are likely involved in this regulation and balance of *Symbiodinium* density. For example, increased cell densities may result from division in the host tissue (Muscatine et al. 1989; McAulley and Cook 1994). On the other hand, decreasing densities could be due to degradation and/or digestion by the host (Titlyanov et al. 1996; Jones and Yellowlees 1997), or release into the water column (Hoegh-Guldberg et al. 1987; Stimson and Kinzie 1991; Fang et al. 1998). Another possibility is that translocation of *Symbiodinium* cells within the same colony is responsible for these apparently stable densities. However, this potential mechanism has not been extensively explored in hermatypic corals (but see Gladfelter 1983).

To gain insight into the spatial and temporal changes of *Symbiodinium* density and division rates within hermatypic corals, we took advantage of the naturally occurring differences in pigment distribution and symbiont cells between the upper and lower sides of *Montipora capitata* plates. We had previously observed that when plates are overturned in the field due to wave action, the pale underside, now exposed to direct illumination, quickly becomes normally pigmented. In *M. capitata* plates, tissue extends into the perforate (porous with an open 3-dimensional mesh-like structure) skeleton, effectively connecting the upper and lower endoderm layers (Fig. 1). In these plates, *Symbiodinium* density is highest in the endoderm of the upper surface, lower in the bottom endoderm layer, and lowest in the reticulate tissue in between these (Kinzie 1993). We experimentally tested two hypotheses that could explain our field observations of the changes in pigmentation and *Symbiodinium* in overturned colonies:

- 1) A – This response to an altered light regime is accomplished without changing *Symbiodinium* cell densities. Instead, cells remain in their original intracolony densities and locations while adjusting their Chl *a* content. **Test:** Conduct *Symbiodinium* cell counts and determine Chl *a* content per cell across segments of overturned *M. capitata* plates.
B – Alternate hypothesis: This response to an altered light regime is accomplished by changes in *Symbiodinium* cell densities in different parts of the colony, with little or no change in Chl *a* content per cell. **Test:** Quantify *Symbiodinium* cell densities across segments of overturned *M. capitata* plates over time.

In the case that Hypothesis 1B is supported:

- 2) A – The observed change in *Symbiodinium* cell densities results from differences in division rates across the colony. **Test:** Measure *Symbiodinium* division rates across segments of overturned *M. capitata* plates over time.
B – Alternate hypothesis: There is little or no change in *Symbiodinium* division rates, rather, changes in density are due to movement of *Symbiodinium* cells through the coral colony. **Test:** Track stained *Symbiodinium* cells in *M. capitata* plates over time.

Materials and methods

Collection and maintenance of *Montipora capitata*

Flat *Montipora capitata* plates (> 200 cm²) with living tissue completely covering both the upper and lower surfaces were collected from the fringing reef surrounding Coconut Island, Kāneʻohe Bay, ʻOahu. Plates were transported to the laboratory and maintained for ~ 3 wks in their original orientation in a shallow black seawater table (minimizing reflected irradiance on the underside of colonies) prior to being utilized in experiments. In some experiments, entire plates were overturned, while in others plates were cut into squares that were used when tissue had healed along the cut edges.

Quantification of chlorophyll *a*

Spatial changes in Chl *a* concentrations were meas-

ured in four overturned *M. capitata* plates by removing four 7.0 mm dia plugs from each coral on Days 0 (the day the plates were overturned) and 30. Three of the plugs were used for Chl *a* determination and one for counts of *Symbiodinium*. Plug lengths were between 5.5 and 11.6 mm, with living tissue extending through them. Extraction and quantification of Chl *a* were performed as in Kinzie (1993), with the exception that each plug was divided into three, equal-length segments. These were designated: “side facing up” (SFU; originally the bottom of the colony), “middle” (MID) and “side facing down” (SFD; the original upper side of the colony). Differences in Chl *a* concentration ($\mu\text{g Chl } a \text{ mm}^{-3}$) were assessed using paired *t*-tests (Day 0 vs. Day 30). The cell counts for the Day 0 and Day 30 plugs were made as in Kinzie (1993).

***Symbiodinium* cell densities and mitotic indices**

For experiments to quantify and localize *Symbiodinium* cells, another three *M. capitata* plates were overturned. One of the plates was sampled for cell counts while the other two were utilized for histological studies, with three replicate cores taken from each plate every 4–5 days for 7 weeks. Cores were sectioned into three segments as described above and cell counts conducted as in Kinzie (1993). Cell densities for each segment per sampling day are expressed as the mean from the three replicates and presented as cells mm^{-3} . Statistical differences were assessed by ANOVA. Sections for histology were made through entire cores to visualize *Symbiodinium* location in each segment of a colony. Cores were fixed, decalcified and embedded in paraffin according to Clark (1981) and Kierman (1990). Four, 7.0 μm -thick H&E-stained sections from each core were randomly selected and *Symbiodinium* cells in the SFU, MID and SFD counted. To ensure objective counts, slide labels were covered. *Symbiodinium* counts made on histological sections of the SFU and SFD were in linear mm of upper and lower endodermal cells, which appeared as rows of cells on the slides (Fig. 1). Densities in MID tissue were from counts made in 1.04 mm^2 fields of view on each section and are normalized to cells mm^{-2} . Note that these do not correspond to areal cell densities as frequently cited in the literature since counts were normalized to dimensions on the paraffin sections

that were only 7.0 μm in thickness.

To determine whether changes in *Symbiodinium* density might be due to differential growth, symbiont division rates were estimated using mitotic index (MI), or the percentage of actively dividing cells (Wilkerson et al. 1988). Samples for MI were drawn from the same tubes used in cell density measurements (see above). A cell was considered to be dividing (undergoing cytokinesis) if it appeared as a doublet with a cell plate. The number of dividing cells in three samples, each of 1,000 cells, was averaged and the resultant percentage taken as the overall MI of that segment (Wilkerson et al. 1983).

Labeling and tracking of *Symbiodinium* cells

Labeling and tracking of *Symbiodinium* cells in *M. capitata* were conducted on seven, 3 \times 3 cm squares obtained from a single colony. In this experiment samples were not overturned. Squares were exposed to a 50 μM 5-bromo-2'-deoxy-uridine (BrdU; Sigma B-9285) solution in seawater with gentle stirring for 48 hours, with seawater and BrdU changed every 4 hours. Following this treatment, one square was randomly selected and

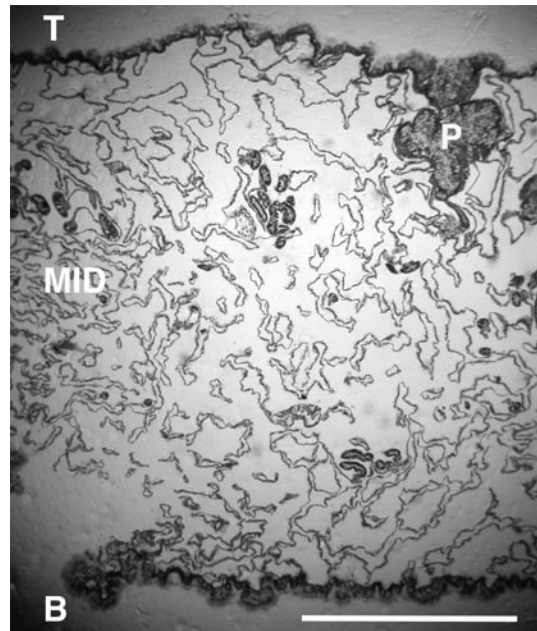


Fig. 1 Cross section through a *M. capitata* plate. Note that tissue penetrates completely through the perforate skeleton (MID) and that the upper (T) and lower (B) endoderm layers are connected. A polyp (P) can be seen in the T endoderm layer. Scale bar (bottom right corner) = 1 mm

sampled immediately (Day 0), followed by sampling of the remaining squares on Days 2, 4, 7, 10, 14, and 21. Squares were fixed, decalcified and embedded as described previously and sectioned at 5.0 μm thickness. Following deparaffinization and rehydration in a graded series of methanol-water baths, immunochemical detection of BrdU was conducted according to procedures outlined in Oinuma et al. (1992) and Uchida and Kaneko (1996). Controls slides were not exposed to anti-BrdU antibodies but received all other procedures. Slides were dehydrated and sealed with coverslips and permanent mounting medium.

Results

Quantification of Chl *a* concentrations verified our field observations. Namely, *Montipora capitata* showed significant changes in Chl *a* across vertical segments of the plates following experimental overturning. Specifically, Chl *a* per unit volume was 3.6X higher in the SFU (former underside of the plate) by Day 30, while being reduced by one-third in the SFD (former top of the plate)

in the same time period, with both changes statistically significant (Table 1).

Hypothesis 1A: Chlorophyll *a* content per *Symbiodinium* cell

Overall, no significant change in Chl *a* content per *Symbiodinium* cell was detected between Day 0 and Day 30 in any of the sections (Table 2). Thus, since Chl *a* per unit volume changed, but Chl *a* content per cell did not, it appears that overturning the *M. capitata* plates promoted shifts in *Symbiodinium* cell densities across the colony.

Hypothesis 1B: Change in *Symbiodinium* cell densities following experimental overturning

Symbiodinium densities comparable to Day 0 were restored 30 days after overturning the *M. capitata* plates (Table 3). Notably, final cell densities were similar to what the starting densities had been on the opposite side of the plate prior to being overturned. To follow the time course of these changes, *Symbiodinium* densities in overturned corals were quantified via direct cell counts and histological examination at intervals over several weeks. The results of this time series confirmed the findings of the

Table 1 *Symbiodinium* chlorophyll concentration in the “side facing up” (SFU; originally the bottom of the colony), “middle” (MID) and “side facing down” (SFD; the original upper side of the colony) of experimentally overturned plates of *Montipora capitata*. Values are in $\mu\text{g Chl } a \text{ mm}^{-3}$

Position in overturned <i>M. capitata</i> plate	Day 0 Mean \pm 1 SD (<i>n</i> =4)	Day 30 Mean \pm 1 SD (<i>n</i> =4)	Paired <i>t</i> -test on differences between Day 30 - Day 0 (<i>n</i> =4)
Side Facing Up (SFU)	0.021 \pm 0.006	0.086 \pm 0.019	<i>P</i> = 0.005
Middle (MID)	0.013 \pm 0.003	0.013 \pm 0.005	<i>P</i> = 0.98
Side Facing Down (SFD)	0.074 \pm 0.021	0.026 \pm 0.012	<i>P</i> = 0.004

Table 2 *Symbiodinium* chlorophyll concentration in the “side facing up” (SFU; originally the bottom of the colony), “middle” (MID) and “side facing down” (SFD; the original upper side of the colony) of experimentally overturned plates of *Montipora capitata*. Values are in $\text{pg Chl } a \text{ cell}^{-1}$

Position in overturned <i>M. capitata</i> plate	Day 0 Mean \pm 1 SD (<i>n</i> =4)	DAY 30 Mean \pm 1 SD (<i>n</i> =4)	Paired <i>t</i> -test on differences between Day 30 - Day 0 (<i>n</i> =4)
Side Facing Up (SFU)	11.33 \pm 5.86	20.39 \pm 21.41	<i>P</i> = 0.389
Middle (MID)	13.27 \pm 5.54	10.32 \pm 3.28	<i>P</i> = 0.539
Side Facing Down (SFD)	6.28 \pm 1.25	8.05 \pm 3.64	<i>P</i> = 0.386

Table 3 *Symbiodinium* density in the “side facing up” (SFU; originally the bottom of the colony), “middle” (MID) and “side facing down” (SFD; the original upper side of the colony) of experimentally overturned *M. capitata* plates. Values are in cells mm^{-3}

Position in overturned <i>M. capitata</i> plate	Day 0 Mean \pm 1 SD ($n=4$)	Day 30 Mean \pm 1 SD ($n=4$)	Paired <i>t</i> -test on differences between Day 30 - Day 0 ($n=4$)
Side Facing Up (SFU)	2,248 \pm 1,005	10,477 \pm 1.215	$P = 0.0004$
Middle (MID)	1,166 \pm 578	1,326 \pm 414	$P = 0.3343$
Side Facing Down (SFD)	11,826 \pm 3,978	2,505 \pm 523	$P = 0.0046$

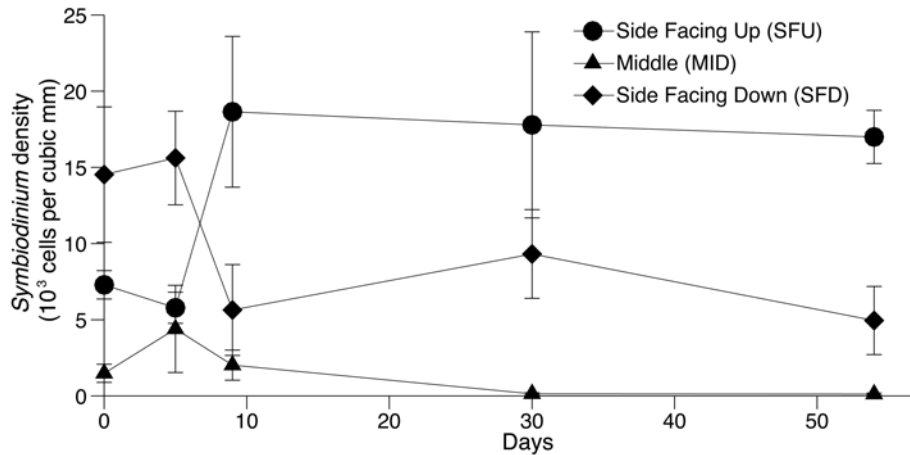


Fig. 2 Time course of changes in densities of *Symbiodinium* quantified by cell counts in plugs from experimentally overturned *M. capitata* plates. The “side facing up” (SFU) was originally the bottom of the colony and the “side facing down” (SFD) was the original upper side of the colony. The “middle” (MID) was the middle third of the plug. Error bars are \pm one standard deviation

previous Day 0 and Day 30 samplings. Specifically, *Symbiodinium* density in the SFU increased $\sim 3X$ by Day 9 before remaining stationary for the remaining 45 days. In contrast, *Symbiodinium* density decreased to less than half the starting density by Day 9 in the SFD. These density changes in both the SFU and SFD were significant (ANOVA; $p < 0.05$), with no significant changes in the MID segment of the colony. These results suggest that the restoration of *Symbiodinium* density occurs within 9–10 days (Fig. 2).

In the histological sections, *Symbiodinium* density in the SFU rose $> 3X$ during the experiment (Fig. 3). This change, however, was not significant due to variation between colonies. On the other hand, *Symbiodinium* density in the SFD declined significantly (ANOVA; $p < 0.05$) from Day 0 to Day 10 and remained low to the end of the experiment. *Symbiodinium* density within the porous skeleton (MID segment) showed the most marked

change with time (ANOVA; $p < 0.0001$). Cell density in this segment was highest on Day 10 and lower both before (Days 0 and 2) and after (Day 37) this time point. The increased *Symbiodinium* density in the MID segment can be seen in Fig. 3.

Hypothesis 2A: *Symbiodinium* mitotic indices

No significant changes in *Symbiodinium* mitotic indices (MI) from the SFD or MID segments of the *M. capitata* plate were identified (ANOVA; $p = 0.84$ and 0.98 , respectively) over the experimental period. Interestingly, MI for the SFU significantly declined from 3.6% on Day 0 to 1.7% by the end of the experiment ($p < 0.05$) (Fig. 4). In other words, the original MI for what was formerly the lower surface of the plate was $> 3X$ higher than that of the side that had been facing up before the plate was experimentally inverted. To determine whether this higher MI in the colony’s shaded underside

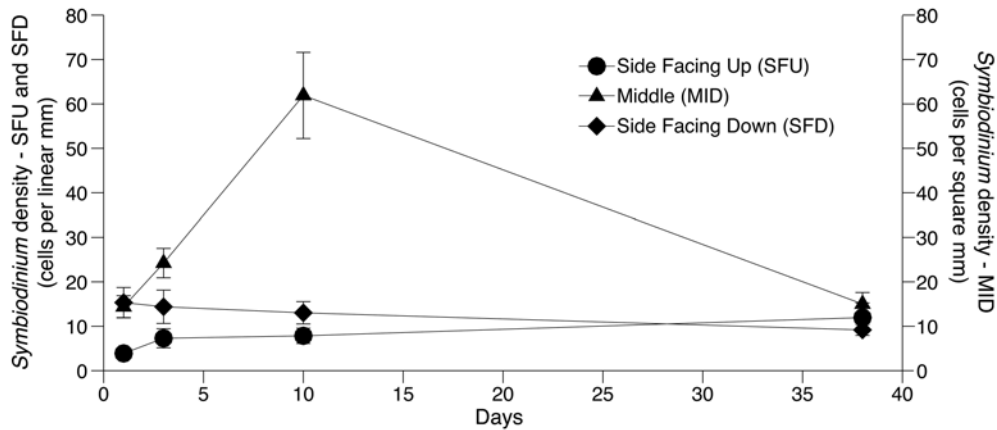


Fig. 3 Time course of changes in densities of *Symbiodinium* quantified by histological sections through the “side facing up” (SFU; originally the bottom of the colony), “middle” (MID) and “side facing down” (SFD; the original upper side of the colony) of experimentally overturned *M. capitata* plates. Note that cell densities for the side facing up (SFU)/side facing down (SFD) and in the middle region are per *linear mm* and per *square mm*, respectively, of coral endodermal tissue on the slides (see text for additional details). Error bars are \pm one standard deviation

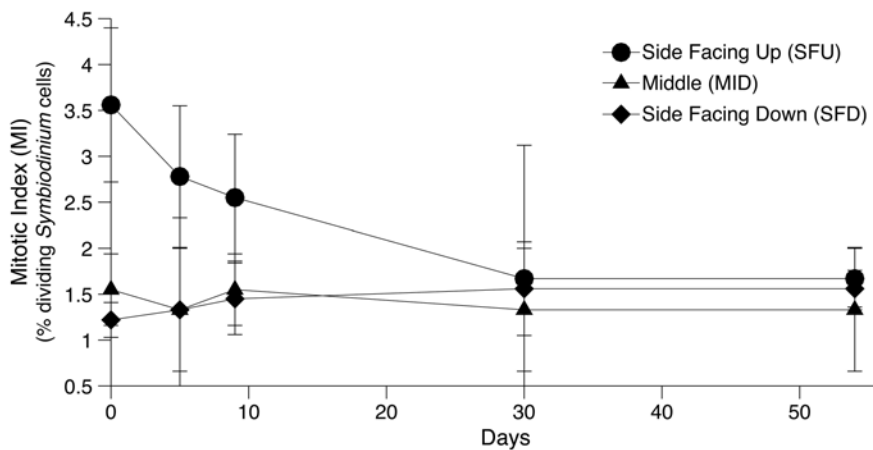


Fig. 4 Time course of *Symbiodinium* mitotic indices (MI) in the “side facing up” (SFU; originally the bottom of the colony), “middle” (MID) and “side facing down” (SFD; the original upper side of the colony) of experimentally overturned *M. capitata* plates. Error bars are \pm one standard deviation

was an experimental artifact, cores were taken from three *M. capitata* plates in the field and preserved immediately using the sampling procedure described above. The MI for *Symbiodinium* cells from the underside of these field-sampled plates was 3.7%, while that of the middle and upper thirds were approximately a third of this and correspond nearly exactly with the analogous segments of the experimental plates at Day 0 (see above).

Although differences in the MI of cells in the SFU were found, they were in the opposite direction predicted by

Hypothesis 2A. This suggests that: a) the restoration of cell density and distribution is not driven by changes in *Symbiodinium* cell division rates (Hypothesis 2A) and b) *Symbiodinium* cells in the underside of plates have greater per capita division rates that are quickly repressed upon exposure to high light levels.

Hypothesis 2B: Movement of *Symbiodinium* cells in *M. capitata*

For the tracking of *Symbiodinium*, the percentage of

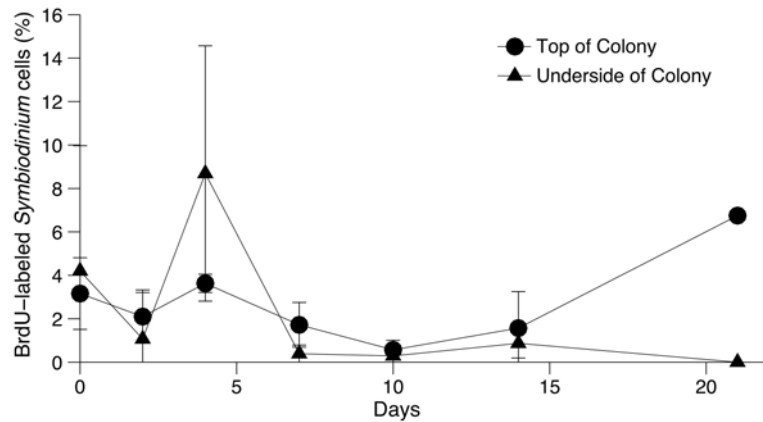


Fig. 5 Time course quantification of BrdU-labeled *Symbiodinium* in the top and underside of “normal” (not overturned) *M. capitata* plates. Error bars are \pm one standard deviation

cells labeled with BrdU was compared in “normal” (not overturned) *M. capitata* plates over 21 days. Mitotic indices of *Symbiodinium* in the plate’s upper and lower endoderm tissue were also quantified. Again, the MI was higher in the lower tissue (as in Fig. 4), although a statistically significant difference was only found 72 hours following BrdU exposure. Initially, *Symbiodinium* cells in the lower endoderm had a slightly higher percentage of BrdU-labeled cells (Fig. 5). This is consistent with the previous finding that *Symbiodinium* on the underside of plates have higher per capita division rates. On Day 4, the percentage of labeled cells doubled in the lower endoderm and showed a similar, but much smaller increase in the upper endoderm (Fig. 5). This is probably due to the production of two daughter cells per each labeled cell following cell division. After this spike, the percent of BrdU-labeled cells was very low in both the upper and lower endoderm. However, by Day 21, the proportion of BrdU-labeled *Symbiodinium* cells in the upper endoderm showed a marked increase (Fig. 5). Since BrdU staining was done only at the start of the 21-day time course, the most parsimonious explanation for the appearance of BrdU-labeled *Symbiodinium* cells in the upper endoderm is translocation through the perforate skeleton from the lower endoderm. This supports Hypotheses 2B – that *Symbiodinium* cells are translocated from the lower (where cell division rates are high) to the upper side of *M. capitata* plates.

Discussion

This study tested two hypotheses regarding the spatial and temporal dynamics of *Symbiodinium* density and division rates within hermatypic corals. We found that the development of dark pigmentation in the initially pale, “new” upper sides of overturned *M. capitata* plates was not due to alterations in Chl *a* concentration per *Symbiodinium* cell, nor could this change in pigmentation be attributed to increased division rates in *Symbiodinium* cells exposed to higher light levels since, in fact, the opposite occurred. Instead, this change appears to be due to translocation of existing *Symbiodinium* cells from one region of the colony to another.

Further evidence for intracolony translocation of *Symbiodinium* is found in the tracking of BrdU-labeled cells in *M. capitata* plates in their natural orientation. For example, *Symbiodinium* in the lower endoderm exhibited a spike in the percent of labeled cells four days following BrdU exposure, suggesting rapidly dividing cells in the lower endoderm are producing daughter cells that also contain BrdU-labeled nucleic acid. By Day 10, few (or none) of these cells were detected in either the upper or lower endoderm layers. On Day 21, however, a marked increase in labeled cells occurred in the upper endoderm, consistent with the hypothesis that the *Symbiodinium* cells had been translocated. While the exact mechanism behind this translocation remains to be elucidated, we suggest

that the cells are most likely transported upwards through the endoderm lining the porous skeletal matrix of the colony rather than through the gastrovascular space or ciliated grooves in the mesenteries since *Symbiodinium* repopulation via such processes would be expected to take a shorter (hours to ~ 1 d) period of time (Gladfelter 1983; Gateño et al. 1998; Rodriguez-Lanetty et al. 2005).

The translocation of *Symbiodinium* cells through a coral colony's porous skeletal matrix may be a common phenomenon since many dominant reef-building genera (*Acropora*, *Goniopora*, *Astreopora*, *Fungia*, *Turbinaria*, *Montipora*, *Porites*, etc) have such skeletons. Other advantages of having tissue that penetrates deeply into the skeleton have also been documented. For example, such an arrangement is thought to facilitate the movement of nutrients or ions important for calcification in the genus *Acropora* (Buchsbaum-Pearse and Muscatine 1971; Gladfelter 1983). Furthermore, deep skeletal tissue can enable some species to survive environmental events that results in significant mortality for others (Jokiel et al. 1993). Such situations include the rapid recovery of perforate coral species in Kāneʻohe Bay, ʻOahu following a severe flood via the 'Phoenix Effect' (Krupp et al. 1993). Additionally, the perforate corals *Porites compressa*, *Fungia scutaria* and *M. capitata* survived for 60 days in darkness (and were eventually repopulated with *Symbiodinium*), while the non-perforate *Pocillopora damicornis* exhibited high mortality after 30 (or fewer) days under identical treatment (Franzisket 1970).

Here, we postulate another potential advantage related to the spatial and temporal dynamics of *Symbiodinium* in corals such as *M. capitata*. For maximum photosynthetic efficiency, *Symbiodinium* cells must be exposed to sunlight. However, while coral colonies and their symbionts receive high levels of sunlight in shallow waters, they also have been shown to exhibit DNA damage when exposed to short wavelength radiation in the process (reviewed by Banaszak 2007). In perforate corals, where *Symbiodinium* can apparently be translocated throughout the colony, cells in more shaded regions may serve as a sub-population experiencing minimal damage to their DNA. In this scenario, newly produced *Symbiodinium* cells could then be moved up into regions of high light, where photosynthesis may be maximized, but at the expense of photo-

damage to the symbionts. The fact that higher per capita division rates were observed in *Symbiodinium* inhabiting the underside of naturally occurring plates is consistent with this hypothesis. Thus, there would be a "conveyor-belt" mechanism operating in the colony, with movement of cells from a "generative zone" with relatively low photosynthesis to the exposed colony surface where sunlight (including short wavelength radiation) is high. Such a situation could also occur in branching corals such as *Acropora*, with deeper regions between branches serving to shade *Symbiodinium* cells. Such a possibility deserves further exploration.

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