Title: Spatial variability of *Symbiodinium* and color in *Montipora capitata* across Kāne‘ohe Bay, O‘ahu, Hawai‘i

Running Page Head: Distribution of *Symbiodinium* and Color

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**ABSTRACT**

Scleractinian corals form mutualistic symbioses with photosynthetic dinoflagellates in the genus *Symbiodinium*. Different individuals may host genetically and functionally distinct symbiont communities that confer varying levels of performance and stress-tolerance to their hosts. However, the factors shaping variable symbiont community composition, which may determine bleaching susceptibility and resistance to future stressors, are poorly understood. To investigate the role of the environment in shaping symbiont communities, colonies of *Montipora capitata* (N = 707) were sampled across reef habitats and depth gradients (0 - 13 m) in Kāne‘ohe Bay, O‘ahu, Hawai‘i, USA, where this dominant reef-builder associates with *Symbiodinium* C31 and/or D1a and also displays two distinct color morphs (brown and orange). Ratios of C31 to D1a determined for each sample by quantitative PCR revealed that colonies dominated by either symbiont occurred throughout the bay with no difference in frequency across regions of the bay, between patch and fringing reef types or among individual reef sites. However, symbiont clade dominance was significantly associated with depth where clade D was more likely to dominate shallow corals and clade C was more likely to dominate colonies at depths > 1.29 m. Colony color morph significantly varied with depth with more orange morphs shallow and brown morphs at depths > 3.64 m. Across depths, brown colonies were more likely clade C-dominated while orange colonies were more likely D-dominated < 2.75 m and C-dominated > 2.75 m. This work reveals that distributions of *Symbiodinium* and color in *M. capitata* result from depth-related abiotic conditions.

**INTRODUCTION**

Coral reefs are among the most biologically diverse ecosystems on the planet, providing essential services such as shoreline protection, serving as vital habitat and functioning as an important source of sustainable revenue as a tourist destination (Barbier et al. 2011). Calcification by hermatypic stony corals in the order Scleractinia builds critical habitat, made possible by the formation of a mutualistic endosymbiosis with photosynthetic dinoflagellates (*Symbiodinium* spp.). Through this symbiosis, the coral host gains the majority of its required carbon as photosynthate translocated from the symbiont (Muscatine & Porter 1977).

Scleractinian corals associate with a diverse array of *Symbiodinium*.Nine clades (A-I) have been described in this genus based on the internal transcribed spacer (ITS) region of nuclear ribosomal DNA (Pochon & Gates 2010). Different symbiont types have characteristic levels of stress-tolerance and physiological optima (Rowan 2004). Some clade C symbionts (e.g. C1) are better able to supply photosynthate to the coral host, yet are less stress-tolerant (Cantin et al. 2009). Conversely, clade D symbionts have shown higher levels of thermal tolerance and photoprotection, but growth rates and photosynthetic efficiency of clade D-dominated corals are often depressed relative to corals associating with other symbiont clades (Little et al. 2004, Rowan 2004).

Most coral species associate with a single symbiont clade, some even with a single type within a clade (LaJeunesse, Bhagooli, et al. 2004, Goulet 2006), but other species are able to host multiple clades concurrently (van Oppen et al. 2001, Rowan 2004). While colonies harboring multiple clades are typically dominated by one clade with background populations of other clades (Silverstein et al. 2012), the presence of heterogeneous mixtures of symbionts implies potential for symbiont shuffling (i.e. a change in the relative abundance of types present) or switching (i.e. an uptake of novel types from the environment) in response to changing environmental conditions (Rowan et al. 1995, Mieog et al. 2007, Jones et al. 2008, Boulotte et al. 2016). Indeed, prevailing environmental conditions may, in part, determine which symbiont is able to establish and maintain dominance in a mixed community (Cunning et al. 2015 *Proc R Soc B*).

Little is known about the environmental factors controlling the spatial distribution of *Symbiodinium* within a species, yet the factors shaping the presence and dominance of symbionts need to be better understood for their implications on colony performance. Irradiance and thermal stress (Rowan et al. 1995, Abrego et al. 2009, Stat et al. 2015) are major drivers of symbiont community differences in corals. *Symbiodinium* of clade D (D1) have shown thermal tolerance not observed in clade C (C1b-c) and can dominate coral colonies in areas that experienced thermal stress (Baker et al. 2016). This is consistent with previous studies of symbiont distribution and sea surface temperatures (SST) in which clade D was correlated with higher SST (Tonk et al. 2013). Similarly, clade C symbionts (C1c) in the Gulf of California performed better in low light at depth than did clade D symbionts (D1) which were more successful in shallow, high light environments, suggesting irradiance plays a significant role in niche-partitioning of *Symbiodinium* (Iglesias-Prieto et al. 2004, Sampayo et al. 2007, Bongaerts et al. 2015).

*Montipora capitata* is a dominant reef-building species inKāne‘ohe Bay that can harbor both clade C (C31) and D (D1a) symbionts (LaJeunesse & Thornhill 2011, Stat et al. 2011, Cunning et al. 2016). However, the factors driving the distribution of these clades in *M. capitata* colonies across environmental gradients are not well described. In Kāne‘ohe Bay, *M. capitata* occurs as distinct brown and orange color morphs (LaJeunesse, Thornhill, et al. 2004). The cause of these different color morphs is unknown but studies suggest that fluorescent protein-like proteins play a role in color development (Matz et al. 1999, Lukyanov et al. 2000). It is unclear what functional differences exist among *M. capitata* color morphs; however around O‘ahu the brown and orange morphs were suggested to possess a specific endosymbiosis with *Symbiodinium* of clades C and D respectively (LaJeunesse, Bhagooli, et al. 2004, LaJeunesse & Thornhill 2011). Both color morphs of *M. capitata* are commonly found in a variety of habitat types in Kāne’ohe Bay, thus providing an excellent study species to explore the factors driving the relative distribution of multiple symbionts and color in a dominant reef-building coral.

Kāne‘ohe Bay is a semi-enclosed bay on the windward side of O‘ahu, Hawai‘i marked with a strong history of anthropogenic and environmental disturbance (Smith et al. 1981, Jokiel 1991) which may contribute to stressful conditions driving variant symbiont assemblages across the bay (Cooper et al. 2011, Stat et al. 2011). The patch and fringing reef systems in Kāne‘ohe Bay are relatively shallow (0 - 17 m) and portions can be exposed during extreme low tides (Bahr et al. 2015), likely contributing to high irradiance levels at shallow depths. Though comparatively shallow relative to other reef systems, light attenuates rapidly with depth due to high turbidity. The bay is often categorized by three regions (north, central and south) based on gradients of decreasing oceanic influence and increasing water residence time. Despite apparently stressful conditions, reefs in Kāne‘ohe Bay have high coral cover (Bahr et al. 2015) and rapid recovery rates from bleaching events (Cunning et al. 2016). The current study was designed to characterize the spatial distributions of *Symbiodinium* clades C and D to investigate the variability in symbioses in the Kāne‘ohe Bay population of *M. capitata*, elucidating patterns of distribution that may explain coral responses to future stress events.

**MATERIALS AND METHODS**

*Study Design and Location*

Individual colonies of *Montipora capitata* in Kāne‘ohe Bay were tagged and sampled between 7 June and 12 August 2016. Colonies at five patch reefs and three fringing reefs were tagged in each of the northern, central and southern regions of the bay along a gradient of decreasing oceanic influence (Bahr et al. 2015) with an additional submerged patch reef south of the Hawai‘i Institute of Marine Biology on Moku o Lo‘e (n = 25 reefs; Fig. 1). At each patch reef, 30 colonies were tagged and sampled: 10 colonies each from the windward slope, reef top and leeward slope. Given the lack of leeward slope on fringing reefs, 20 colonies were tagged and sampled at each site: 10 colonies each from the reef top and slope. At each collection point, colony depth was individually recorded using a depth gauge. At the reef tops of both the patch and fringing sites most colonies were ≤ 1 m depth, and along the windward and leeward slopes, colonies were tagged at 1 - 13 m depth. At reefs lacking sufficient colonies deeper than 5 m, additional colonies were sampled to increase the replication of colonies found at a relatively deeper environment. In total, 16 patch reefs and 9 fringing reefs were sampled across Kāne’ohe Bay resulting in 25 collection sites and a sample size of 707 colonies.

*Sample Collection and Processing*

Ten weights with attached floats were randomly thrown from the surface across a distance of approximately 20 m on each reef area (top and slope(s)). The closest colony of *M. capitata* in proximityto each float was tagged and sampled. *In situ* photographs with an included scale bar and color standard were taken of each colony to later assess the size and color morph of each colony (Fig. 2). A tissue biopsy was collected from each colony from the tip of a branch (~ 4 - 5 cm) located at the top of the colony and placed in 500 μL DNA buffer (5M NaCl, 0.5M EDTA) with 1% sodium dodecyl sulfate. DNA was extracted from each sample biopsy following a CTAB-chloroform protocol (dx.doi.org/10.17504/protocols.io.dyq7vv). The relative abundances of clades C and D *Symbiodinium* within each sample were assayed using quantitative PCR assays targeting actin loci specific to each clade (Cunning & Baker 2013). All samples were assayed in duplicate 10 μL reactions for 40 cycles on a StepOnePlus platform (Applied Biosystems) with a fluorescence threshold of 0.01 and a baseline interval of cycles 15 - 22. The ratio of clade C to D in each sample was calculated by the formula , where CT(C) and CT(D) are the threshold cycles for the clade C and D reactions and KCD is the ratio of gene copy number per cell between clades C and D (Mieog et al. 2007). This ratio was normalized for fluorescence intensity and locus gene copy number (Cunning et al. 2016). Symbiont clades detected in only one technical replicate were not considered as present in a colony. Based on the proportion values of clades C and D, the dominant symbiont type in each colony was determined. If a colony harbored both symbiont clades, designated as a heterogeneous mixture, the clade present in higher proportion was noted as CD or DC accordingly.

*Data Analysis*

The differences in proportion of clades C and D present in colonies dominated by each clade and color morph were investigated using Chi-Squared analyses. Chi-Squared tests were then used to assess differences in the proportion of C- vs. D-dominated colonies, orange vs. brown colonies and one vs. two symbiont clade assemblages between bay regions (northern, central and southern), reef types (patch vs. fringe) and each individually sampled reef. To estimate the probability of C- vs. D-dominance and orange vs. brown coloration as a function of depth, logistic regressions of generalized linear models were used. Depth was corrected for differences in mean sea level using daily tide tables for Moku o Lo‘e, Kāne‘ohe Bay at 6-minute intervals. The effects of depth and location (bay region, reef type and reef ID) on symbiont dominance and colony color morph were tested based on generalized linear models. Spatial autocorrelation among reefs in the composition of color morph and dominant symbiont associates (based on Bray-Curtis dissimilarity) was assessed by a Mantel Test after a Multinomial Logistic Regression was performed to discount the influence of depth on the spatial distribution of the interaction (Lenth 2016). A final Chi-Squared analysis was run on the interaction of colony color morph and symbiont dominance as a function of location (i.e. bay area). All analyses were performed in R v.3.2.2 (R Core Team 2016).

**RESULTS**

*Community Composition*

Quantitative PCR on 707 colonies of *Montipora capitata* detected *Symbiodinium* clades C and D present both in heterogeneous mixtures and as a single clade exclusively. Across all samples, 53 % exclusively contained clade C, 1.2 % exclusively contained clade D and 45.8 % contained a mixture of clades C and D. Clade C was prevalent across all samples, being present in 98.7 % of colonies and the dominant symbiont in 61 % of colonies. In 86.6 % of C-dominated colonies, clade C was the only symbiont present. In striking dissimilarity, only 3.3 % of D-dominated colonies harbored clade D *Symbiodinium* exclusively (Fig. 3). However, when clade D was present in a colony, it was almost always in abundance > 80 %, demonstrating that presence of clade D often indicates a D-dominated colony (Fig. 4). Across the 707 sample colonies, 407 colonies were orange and 300 colonies were brown. Clade C-dominance was observed in 89 % of brown colonies and 41 % of orange colonies (*p* < 0.001; Fig. 4).

*Spatial Distribution*

The proportions of colonies that were C- or D-dominated did not differ among regions of the bay (*p* = 0.14) or reef types (*p* = 0.37) alone. However, the proportions of C- and D-dominated colonies differed between patch and fringing reef types when considering the influence of depth (p < 0.01). The proportions of orange and brown colonies also differed between reef types when considering the influence of depth (p < 0.05). When the influence of the submerged reef south of the Hawai‘i Institute of Marine Biology was excluded from the analysis, bay region proved to be more influential on the likelihood of being a C- or D-dominated colony, yet the relationship was not significant (*p* = 0.06). After adjusting for the influence of colony depth, bay region was a significant predictor of a colony’s likelihood of being C- or D-dominated and brown or orange in coloration concurrently (*p* < 0.01; Fig. 5). D-dominated brown colonies were more abundant in the southern (*n* = 18) bay area than they were central (*n* = 10) and northern (*n* = 5) regions, though this trend is potentially due to a small sample size with only 33 brown colonies dominated by clade D.

Depth proved to be the significant driving factor for symbiont dominance (*p* < 0.001) and color morph (*p* < 0.001) among colonies of *M. capitata*. The probability of a colony being C-dominated increased at depths below 1.29 m, while D-dominated colonies were more common at depths above 1.29 m. A higher probability of a colony being orange was observed in colonies at depths < 3.64 m, whereas brown color morphs dominated at depths > 3.64 m. Brown colonies were more likely to be dominated by clade C *Symbiodinium* across all depths, but orange colonies were more likely to be dominated by clade D at depths above 2.75 m and clade C at depths deeper than 2.75 m (*p* < 0.001; Fig. 6).

**DISCUSSION**

The symbiont composition of sample colonies existed as either a heterogeneous mixture of clades C and D or as one clade exclusively. In the 707 sample colonies, clade C was present in 98.7 %, while clade D was found in only 46.8 %. When clade D was present, however, it typically dominated the symbiont community (Fig. 4). Of the nine colonies that exclusively harbored clade D, five colonies showed amplification of clade C in one of the technical qPCR replicates, suggesting likelihood that clade C was present in low abundance but at a concentration below the detection threshold of qPCR and the conservative estimate of symbiont presence applied in this study. Overall, this indicates that clade D almost never occurred without clade C, yet clade C often occurred exclusively, potentially indicating a competitive dynamic between clades in which clade C is an ideal symbiont and clade D serves as an opportunist.

Symbiont association and colony color in *Montipora capitata* across Kāne‘ohe Bay showed strong depth-dependent distributions (Fig. 6). Shallow colonies were more likely to be orange and D-dominated whereas deeper colonies were more likely to be brown and C-dominated. Across depths, brown colonies were never more likely to be D-dominated, yet orange colonies were more likely D-dominated shallower than 2.75 m and C-dominated deeper than 2.75 m. This distribution was observed across all sampled reefs, indicating that the factors driving these patterns in Kāne‘ohe Bay exist along a depth gradient rather than a geographic distribution. No significant spatial differences were observed across regions of the bay, individual reefs or reef types, consistent with the lack of spatial variation in symbiont prevalence previously observed across sites and regions (Stat et al. 2011). Depth partitioning supports observations that habitat depth influences bathymetric zonation of coral symbionts between shallow, high irradiance environments and deep, low irradiance environments (Frade et al. 2008b, Finney et al. 2010).

Colonies were more likely to be orange in shallow reef areas but were rare at depths below 3.64 m, where brown colonies were more prevalent. The prevailing division of color has previously correlated with symbiont community differences, consistent with our findings of orange colonies more likely being D-dominated and brown colonies more likely being C-dominated (LaJeunesse, Bhagooli, et al. 2004, LaJeunesse & Thornhill 2011). However, color differences in this species, particularly their distributions, are understudied overall. Previous studies suggest color results from phenotypic plasticity in fluorescent protein-like proteins (Kelmanson & Matz 2003, Dove 2004) while other studies determined depth-related abiotic conditions shape color morph (Frade et al. 2008a).

We argue the latter proves more influential in shaping distributional patterns of *M. capitata* across Kāne‘ohe Bay (Dove et al. 2006). For example, at depth, brown morphs of *Porites astreoides* were observed more frequently than green morphs, suggesting acclimation to low-light environments (Gleason 1993). This phenomenon may be pertinent to *M. capitata*,which exhibited a comparable pattern where brown morphs were more common at depth where light intensity was likely reduced and orange morphs were more common in shallow environments probably exposed to higher light intensity. Consequently, it is hypothesized that orange morphs may have some photoprotective properties (Salih et al. 2000) contributing to the prevalence of this color morph in shallow depths, but further investigation of this potential mechanism of color variation in *M. capitata* is needed.

Photoprotection is common in habitats exposed to high light levels and is thought to help coral resist photoinhibition in high light conditions (Jones & Hoegh-Guldberg 2001, Dove 2004). Several photoacclimatory mechanisms have been suggested including adjustments of photosynthetic pigments to avoid oxidative stress from excess light energy (Titlyanov 1981). Non-photochemical quenching (NPQ), the dissipation of excess energy from excited-state chlorophylls, causes carotenoid pigments to adopt harvesting or protective forms depending on environmental conditions (Müller et al. 2001) and may contribute to adaptations among scleractinian corals in habitats with different light intensities. Given the significant association observed between symbiont clade and color morph in *M. capitata* across a narrow depth range, there exists a possibility for NPQ to play a role in persistence of this species in Kāne‘ohe Bay. Theoretically, clade D symbionts and/or orange color morphs found in shallow environments would have a higher capacity for NPQ, though no studies have yet investigated this.

Symbiont association in *M. capitata* was highly depth-dependent where D-dominated colonies were more prevalent in shallow environments and C-dominated colonies were more prevalent at greater depths, suggesting abiotic factors also drive this pattern.Physical conditions in Kāne‘ohe Bay are observably variable within a narrow depth-range. Across broader geographic ranges, however, some physical gradients such as daily mean temperature and sedimentation are more consistent. (Ritson-Williams & Gates 2016a, Ritson-Williams & Gates 2016b) The highest values of PAR at 2 m depth were recovered in the southern region of the bay (Ritson-Williams & Gates 2016c), which may contribute to the greater number of brown colonies harboring clade D in this region (Fig. 5) supporting the notion that clade D symbionts are possibly more tolerant to high light environments (Finney et al. 2010). Symbiont association in *M. capitata* switched from a greater prevalence of clade D to clade C at a transition depth of 1.29 m. As Kāne‘ohe Bay is quite turbid (Grigg 1965), this shallow threshold suggests that depth stratification of light intensity might be a common driver of distribution among symbiont association in *M. capitata*, though the depth of transition would depend on local abiotic conditions.

Light attenuation with increasing depth in the photic zone has been considered the major environmental factor influencing symbiont association and efficiency in coral endosymbioses (Rowan et al. 1995). Differences in photosynthetic responses to environmental conditions are known to exist as photoinhibition and photoprotection in clades C and D respectively (Salih et al. 2000). Such a variable response confirms the hypothesis that different clades of *Symbiodinium* adapt to particular light intensities (Iglesias-Prieto et al. 2004) and supports the idea that habitat partitioning of the symbiont community composition exists along a depth-mediated light gradient (Iglesias-Prieto & Trench 1994) whether this be total irradiance or spectral niches as some studies suggest (Frade et al. 2008a). While irradiance is the most probable candidate, temperature, freshwater lensing and suspended particles all potentially influence coral symbioses as well. Therefore, we suggest investigation of these parameters across depths in Kāne‘ohe Bay to determine the likelihood of each to contribute to the significant patterns of distribution.

Quantitative PCR analysis of *M. capitata* colonies across Kāne‘ohe Bay showed that spatial variability of *Symbiodinium* is depth-dependent. There was also a strong influence of depth on the distribution of colony color morph. No significant spatial distributional patterns arose from different reefs, reef types or areas of the bay when considering the dominance of one clade or color over another. Portions of the reefs in Kāne‘ohe Bay can be quite shallow (< 0.5 m) at low tide and are probably exposed to high temperatures and irradiance during summer months. Corals associating with clade D dominate this highly variable, shallow environment, and because clade C and D symbionts have different physiological tolerances, distributional patterns across depths may serve as an adaptive mechanism to improve symbiotic efficiency in variable environmental conditions.

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**FIGURE CAPTIONS**

Fig 1. Collection reef locations in Kāne’ohe Bay, O’ahu, Hawai’i, USA

Fig. 2. *Montipora capitata* colonies of both color morphs: orange (left) and brown (right). Photo credit: Raphael Ritson-Williams

Fig. 3. Proportion of occurrence of *Symbiodinium* clades C and D in *Montipora capitata* colonies per dominant symbiont clade

Fig. 4. Percentage of clade D in all colonies of *Montipora capitata*. Bar colors indicate colony color morph

Fig. 5. Latitudinal geographic distribution of *Symbiodinium* and color morph in *Montipora capitata* across Kāne’ohe Bay, O’ahu, Hawai’i, USA

Fig. 6. (Top) Bars indicate the proportion of clade-dominance in all colonies grouped by 1m depth intervals. Line indicates the probability of clade D-dominance as a function of depth. (Middle) Bars indicate the proportion of occurrence of each color morph in all colonies grouped by 1m depth intervals. Line indicates the probability of occurrence of the orange color morph as a function of depth. (Bottom) Probability of clade D-dominance for all colonies of each color morph as a function of depth