**INTRODUCTION**

Coral reef habitats are among the most biologically diverse ecosystems on the planet. They provide essential services such as protecting the shoreline, serving as vital habitat for fish and other organisms and acting as a tourist destination with economic value. The unique growth forms that provide the structural framework and rugosity of coral reefs are due to the calcification of stony corals in the order Scleractinia. Such calcification is made possible by the formation of a mutualistic endosymbiosis with photosynthetic dinoflagellates in the order Symbiodinium, in which the coral host relies on photosynthate from the symbiont (Baker 2003). Nine divergent clades (A-I) exist among *Symbiodinium* spp. based on the internal transcribed spacer (ITS) region on nuclear ribosomal DNA (Stat et al. 2011). This diversity results from factors such as host species, depth and irradiance (Rowan et al. 1995). Bleaching, the stress-induced breakdown of the symbiosis via the mechanism of symbiont expulsion, has become an increasingly common phenomenon resulting from climate change. Elevated temperatures and higher irradiance are the more devastating anomalies that cause bleaching (Weis 2008). By understanding the symbiont community composition, the susceptibility to climate change may be more readily understood.

*Symbiodinium* clades C and D are the dominant clades observed in Kāne’ohe Bay and are both observed in *M. capitata*. Each symbiont clade has characteristic levels of stress-tolerance and physiological optima (Boulotte et al. 2016). Clade D, for instance, has shown higher levels of thermal tolerance, yet growth rates of clade D-dominated corals can be depressed, posing a cost-benefit analysis of harboring different symbiont strains (Stat et al 2013). Not much is known about the environmental factors contributing to symbiont variation and distribution however. Evidence of biogeographic patterns across latitudinal gradients, inshore and offshore reefs and even within the same reef environment exists, and the factors driving these patterns are important for understanding coral response to climate change (LaJeunesse et al. 2004; Garren et al. 2006).

*M. capitata* is atypical in its harboring of both *Symbiodinium* clades C and D because the majority of coral species tend to be quite specific, relying on a single symbiont type (Goulet 2006). While colonies are typically dominated by one clade over the other, the presence of heterogeneous mixtures of multiple symbionts suggests the potential for symbiont shuffling or switching in response to climate change (Jones et al. 2008). Few studies have investigated the patterns of association between the two symbionts, especially when considering differences in habitat. *M. capitata* is an essential study species in Hawai’i because it is a dominant reef-builder on the local reefs with the ability to host multiple symbionts.

The patch reef and fringing reef systems of Kāne’ohe Bay are quite shallow; some sections of the reefs can be exposed during low tides (Bahr et al. 2015). Shallow depths, along with restricted circulation throughout the bay, pose negative implications for thermal stress, which has been observed in successive bleaching events in 2014 and 2015. Despite the seemingly intolerable physiognomies of Kāne’ohe Bay, there exists high coral coverage and rapid recovery rates from stress events. This study aimed to characterize the spatial patterns of *Symbiodinium* clades C and D to investigate the potential stress-response of the Kāne’ohe Bay population of *M. capitata* in light of recent bleaching anomalies.

**METHODS**

*Study Design and Location*

Individual colonies of *Montipora capitata* were tagged and sampled to determine the spatial variability of *Symbiodinium* clades C and D found in colonies from different habitats. All corals were sampled from Kāne’ohe Bay, located on the east side of O’ahu in Hawai’i, USA. Corals were tagged with medium-sized yellow cattle tags throughout Kāne’ohe Bay (Fig. 1). Colonies at five patch reefs and three fringing reefs were tagged in each of the northern, central and southern regions of the bay with an additional submerged reef south of the Hawai’i Institute of Marine Biology. At each patch reef, 30 colonies were tagged; 10 colonies each from windward slope, top and leeward slope with depth recorded using a depth gauge. Given the lack of leeward slope on fringing reefs, 20 colonies were tagged at each site; 10 colonies each from the top and slope. At the tops of the patch reefs and the fringe sites most colonies were between 0 and 1 meter depth. Along the windward and leeward slopes, colonies were tagged randomly at a depth from 0.5 meters to 15 meters. Reefs lacking colonies from depths greater than 5 meters were re-visited and five additional colonies were sampled. Depth was later adjusted according to differences in mean sea level using NOAA’s daily tide tables for Moku o Lo’e, Kāne’ohe Bay at 6-minute intervals. In total, 16 patch reefs and 9 fringing reefs were sampled across Kāne’ohe Bay resulting in a sample size of 707 colonies. Tagging, photographing and sampling of colonies took place between 7 June 2016 and 12 August 2016.

*Sample Collection and Processing*

Ten weights with attached floats were randomly cast from the surface across a distance of approximately 20 meters on each reef area (top and both slopes). The closest colony of *M. capitata* in proximityto each float was tagged and sampled. Each sample consisted of a small branch fragment (~4-5cm) taken from the tip of a branch located at the top of the colony. *In situ* photographs with an included scale bar and color standard were taken of each colony. The coral fragment was subsampled for a tissue biopsy shortly after collection (never greater than 1.5 hours), which was placed in 500μL DNA buffer (5M NaCl, 0.5M EDTA) with 1% sodium dodecyl sulfate (SDS). The remaining fragment was immediately frozen in liquid nitrogen and archived at -80°C in the laboratory. DNA was extracted from each sample biopsy following a modified CTAB-chloroform protocol (dx.doi.org/10.17504/protocols.io.dyq7vv).

*Symbiodinium Community Analysis*

Quantitative PCR (qPCR) was used to analyze the symbiont community of each collected sample. Based on amplification of the internal transcribed spacer region (ITS2), sequences of *Symbiodinium* clades C and D resulted from existing clade-level primers and probes (Cunning et al. 2013). All samples were assayed with primers of both clades C and D in duplicate 10μL reactions on a StepOnePlus platform (Applied Biosystems) for 40 cycles. Parameters were set at a fluorescence threshold of 0.01 and a baseline interval of cycles 15-22. The StepOnePlus software produced the target symbiont ratio of clade C to D in each sample, normalized for fluorescence intensity and locus gene copy number. Symbiont clades detected in only one qPCR reaction were not considered. The proportion of clade C dominance was calculated from the clade C to D ratio by the formula [(C:D)/(C:D+1)]. The resulting proportion of clade D dominance was then calculated by the formula 1-[(C:D)/(C:D+1)]. Based on the proportion values of clades C and D, the dominant symbiont type was determined. If a colony possessed both symbiont clades, designated as a mixture, the clade present in higher proportion was noted as CD or DC accordingly.

*Data Analysis*

Chi-Squared tests were used to assess differences in colony color morph, dominant symbiont clade and symbiont community mixtures between reef areas, bay areas and reef types. To estimate the probability of occurrence of color morph, dominant symbiont and symbiont community mixture as a function of increasing depth, logistic regressions of generalized linear models were used. Depth was standardized according to differences in mean sea level using daily tide tables for Moku o Lo’e, Kāne’ohe Bay at 6-minute intervals.

Distribution of c to d:

* across habitat types,
* across bay regions,
* across depths
* in different color morphs