**INTRODUCTION**

Coral reef habitats are among the most biologically diverse ecosystems on the planet. Large assemblages of coexisting organisms form these ecologically crucial environments, which provide cumulative services. Such services include, but are not limited to, protection of the shoreline, providing essential habitat for fish and other organisms and serving as a tourist destination for economic growth. The rugosity and unique growth forms that provide the structural framework characteristic of coral reefs is due to the calcification of stony corals in the order Scleractinia. Such calcification is made possible by the formation of an endosymbiosis with photosynthetic dinoflagellates of the order Symbiodinium. Nine clades (A-I) exist among *Symbiodinium spp.* based on the internal transcribed spacer (ITS) region on nuclear ribosomal DNA (Stat et al. 2011). Such a diversity results from a variety of factors including host species, depth, irradiance Bleaching has become an increasingly common phenomenon resulting from climate change, and results in the breakdown of this symbiosis via the mechanism of symbiont expulsion. By understanding the symbiont community composition, the effects of climate change can be more readily understood.

Kāne’ohe Bay is the largest bay on the island of O’ahu. Located on the eastern side of the island, Kāne’ohe Bay is characterized by an extensive fringing reef and patch reef system throughout. A history of disturbance by means of development, runoff, dredging and pollution has caused Kāne’ohe Bay to serve as a non-ideal habitat for coral survivorship. The reefs are quite shallow; some sections can be exposed during low tides. This can have negative implications for thermal stress, which has been observed in successive bleaching events in 2014 and 2015. Despite the seemingly intolerable nature of the bay, there exists high coral coverage (~80%) and rapid recovery rates from stress events. However, what it has in survivorship, Kāne’ohe Bay lacks in diversity. *Porites compressa* and *Montipora capitata* are the two dominant reef building coral species that make up a significant proportion of the coral richness in the predominantly homogeneous bay. Consequently, they serve as crucial study species for the symbiont community.

*Symbiodinium* *spp.* clades C and D are the dominant clades observed in Kāne’ohe Bay. Clade C is the more idealistic symbiont because of its delivery of more nutrients to the coral host comparatively. In non-idealistic environments, particularly thermally stressful habitats, clade D is more beneficial. *Symbiodinium spp.* in clade D have shown more resistance to thermal stress, yet the contributions to coral health and growth are less dramatic. Not much is known about the environmental factors contributing to symbiont variation however. This study aimed to understand the spatial variability of symbiont clades in *Montipora capitata* across Kāne’ohe Bay given its history of disturbance and non-idealistic conditions.

A large sample size of randomly selected colonies at a variety of bay areas, reef areas and depths served as a representative sample of the Kāne’ohe Bay population of *Montipora capitata*. Quantitative PCR was used to analyze the symbiont community composition for each colony by amplification of the internal transcribed spacer (ITS2) region. In doing so, the implications toward bleaching susceptibility and recovery of a dominant reef-building coral are more firmly understood.

**METHODS**

*Study Design and Location*

Colonies of *Montipora capitata* across Kāne’ohe Bay, O’ahu, Hawai’i, USA were tagged and sampled in order to analyze the spatial variability of *Symbiodinium* clades. Corals were tagged on patch reefs and fringing reefs in the northern, central and southern regions of Kāne’ohe Bay (Figure 1 - map). Colonies at five patch reefs and three fringing reefs were tagged in each region of the bay with an additional patch reef in the southern region. At each patch reef, 30 colonies were tagged; 10 colonies each from windward slope, top and leeward slope. Along the windward and leeward slopes, colonies were tagged on a depth gradient. Given the lack of leeward slope on fringing reefs, 20 colonies were tagged at each site; 10 colonies each from the top and slope. The slope was again tagged on a depth gradient. Fieldwork sampling of colonies took place between 7 June 2016 and 12 August 2016. Reefs lacking colonies from greater than 5m were re-visited and five additional colonies were sampled. In total, 16 patch reefs and 9 fringing reefs were sampled across Kāne’ohe Bay resulting in a sample size of n=707 colonies (1 colony lost). Fragments from each colony were frozen in liquid nitrogen and archived for future use in DNA extraction and collaborative efforts analyzing biogeochemistry and energetics in Kāne’ohe Bay.

*Sample Collection and Processing*

Ten weights with attached floats were randomly deployed along a depth gradient at each area of the reef. The closest colony of *Montipora capitata* in proximity to each float was tagged and a small branch fragment (~4-5cm) was removed. *In situ* photographs were taken of each colony for color morph and size analysis by visual assessment. Tissue biopsies were taken from each collected fragment and placed in 500μL DNA buffer (5M NaCl, 0.5M EDTA) with 1% sodium dodecyl sulfate (SDS) and the remaining fragment was frozen in liquid nitrogen to be stored at -80°C as an archive for future analyses. 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 internal transcribed spacer (ITS2),clade C and D *Symbiodinium* sequences resulted from existing clade-level primers and probes. All samples were assays of both clade C and D *Symbiodinium* in duplicate 10μL reactions on a StepOnePlus platform (Applied Biosystems) for 40 cycles. Parameters were set for a fluorescence threshold of 0.01 and a baseline interval of cycles 15-22. The StepOneplus software produced the target symbiont ratios of clade C to D in each sample, normalized for fluorescence intensity and locus gene copy number. Symbiont clades detected in fewer than both duplicate qPCR reactions 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. Whether one clade of *Symbiodinium* or both comprise the symbiont community was also established from the proportion values. If a colony possessed both symbiont clades, the more abundant clade 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 composition between reef areas, bay areas and reef types. To estimate the probability of each color morph, dominant symbiont and symbiont community mixture to occur as a function of increasing depth, logistic regressions of generalized linear models were used. MORE TO BE ADDED AS ANALYSIS CONTINUES.