HABITAT AND BLEACHING IN THE FORAMINIFERAN *PENEROPLIS PERTUSUS*

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Abstract. The effects of human activities on the earth's environment have gained increasing attention in recent years. With coral reefs declining worldwide, efficient tools for assessing reef health are more important than ever. The species of larger foraminifera known as *Peneroplis pertusus* share key characteristics with reef building corals. By examining the populations' natural distribution along with the abiotic factors affecting bleaching, a better understanding of reef systems as a whole is achieved. In this study, *P. pertusus* was collected from ten different sites on a fringing reef in Moorea, French Polynesia. Collected from coral rubble at one, two, and three meters depths, they were analyzed for abundance, size, and extent of bleaching. Light experiments were used in the laboratory to determine response to increased solar radiation. One-way statistical analysis, along with the Wilcoxon test found no strong correlation between depth and percent bleaching. A difference between individual size and percent bleaching was found and a natural population dynamics are presumed to occur on Moorea. Light experiments found increased bleaching in *P. pertusus* showing increased solar radiation to be a factor in bleaching.

Key Words. Larger foraminifera, Peneroplis pertusus, bleaching, distribution, Mo'orea, French Polynesia

INTRODUCTION

The coral reefs found in tropical and subtropical waters play a vital role in human society. More than 50% of the world's population lives within 100km of the ocean (Vitousek *et al.* 1997) and many of those people depend on reef ecosystems for their livelihood. Coral reefs are a valuable economic resource, providing income through tourism and fishing as well as lending protection to coastal habitats. Known as highly diverse and productive environments, coral reefs provide a place of continuing scientific discovery in the form new drugs and biochemicals (Hoegh-Guldberg 2006). Unfortunately coral reef habitats are in decline worldwide (Talge 2003). While susceptible to natural stressors such as disease and hurricanes, the increasing rate at which they are deteriorating has gained interest in recent years.

The impact of human activities on the earth's oceans has been well documented. Alterations to the earth's carbon and nitrogen cycles affect ocean chemistry and ecology. Primarily through fertilizer production, humans have doubled the annual input of biologically available nitrogen to terrestrial ecosytstems (Hallock 2000). Nutrification of estuaries and coastal waters can occur when a significant percentage of fixed nitrogen washes into the ocean (Vitousek *et al.* 1997). The increase of CO_2 in the earth's atmosphere is the best-documented global change (Vitousek *et al*. 1994). Achieved primarily through the burning of fossil fuels, this increase will be responsible for more than half the expected global warming in the next century (Vitousek 1994). Concentrations of greenhouse gases are expected to double over pre-Industrial Revolution concentrations in the early 21st century (Hallock 2000) and ocean temperatures have risen roughly 1°C in the past 100 years (Hoegh-Guldberg 2006). Depletion of ozone in

the atmosphere is linked to an increase of more damaging ultraviolet radiation to reaching earth's surface (Kerr *et al.* 1993). All of these changes can have a profound effect on fragile coral reef communities that are already living at the extremes of their habitat requirements (Hoegh-Guldberg). With these trends from human impact showing no signs of slowing (Hallock 2000, Hallock *et al.* 2003), efficient tools for assessing reef condition are gaining more interest.

Larger benthic foraminifera have been found to be good indicators of reef health (Renema 2006). Foraminifera require many of the same environmental factors to survive as coral (Renema 2006). Larger foraminifera share three distinct traits with reef building corals. Both are producers of calcium carbonate, are reliant on algal endosymbionts for growth and test formation, and both are experienced bleaching events in the past decades (Hallock *et al. 2006)*. A main difference between the groups is the apparent mechanism of bleaching. Coral bleaching occurs through the expulsion of symbionts and has been shown to most strongly correlate to elevated water temperature (Hallock *et al.* 2006). Bleaching in foraminiferan happens when damaged symbionts are digested and is induced through high intensity solar radiation (Hallock *et al.* 2006).

Hallock *et al*. (2003) points to the key characteristics that give foraminifera value as a bioindicators: 1) Their comparatively short life spans compared with corals, allows for comparisons between long and short-term trends 2) They are relatively small and abundant which allows for inexpensive collection and significant sample size 3) Collection and sampling has minimal impact on the reef environment.

Sensitivity to change is an important attribute of a bioindicator. Studies have shown that symbiont bearing foraminifera respond to photo-oxidative stress by bleaching within hours or days (Hallock *et al*. 2006), allowing for significant finding in short term studies. On a larger scale, nutrient influx causes a shift in assemblages from larger symbiont bearing foraminifera to smaller fast growing heterotrophic taxa (Cockey *et al.* 1996).

Peneroplis pertusus is a species of larger foraminifera that can be found abundance on the fringing reef of Mo'orea French Polynesia. It hosts the red algal symbiont *Porphyridium* (Hawkins *et al*. 1990). Most studies on distribution have been done on empty tests and habitats of only a few genera have been investigated in detail (Hohenneger *et al.* 1998). This study examines the distribution of *P. pertusus* along a three-meter depth gradient. The investigation of distribution by water depth represents a "typical ecological complex-gradient" (Hohenegger *et al.* 1995). Factors included in this gradient are topography, light penetration, hydrodynamics, and temperature (Hohenegger *et al.* 1995)

It is important to understand parameters effecting distribution in order to relate it to reef conditions as a whole. The identification of patterns in diversity and assemblage require extensive data (Tappan et al 1998) and this study hopes to contribute to that. The objective is to gather data on the abiotic factors that are effecting the populations of *Peneroplis pertusus*. Examination of distribution, habitat, and the extent of bleaching in the population's natural environment along with experimental manipulations were used to gain a better picture of their role in Mo'orea's reef system.

METHODS

Study Site

Mo'orea is a high volcanic island encircled by a barrier reef. Enclosed in the barrier reef is a shallow back reef between 500 and 100 meters in width (Venec-Peyre 1990). A fringing reef encircles much of the islands coastline including Cook's Bay. The site is located in Cook's Bay, Mo'orea at the University of California's Gump Station (Figure 1). It was chosen for the

accessibility it provides to a suitable fringing reef. The coastline also offers a basic topography that allowed for straightforward site mapping and individual site distinction.

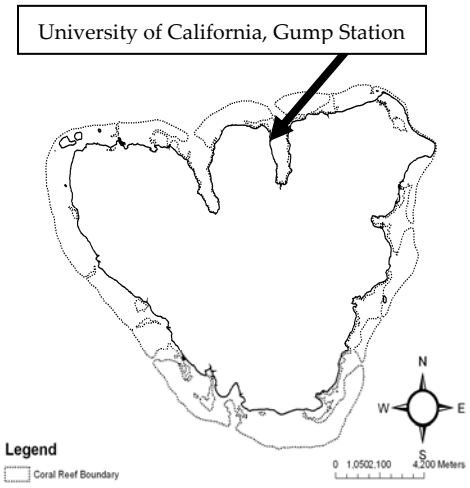

Figure 1. Map of Mo'orea showing Gump Station located in Cook's Bay.

The station's 81-meter coastline provides two distinct fringing reef topographies. The southern 27 meters of property consists of a reef with a sharp depth gradient, reaching three meters between 3-6 meters offshore. The remaining northern 54 meters has a shallow sloping reef reaching three meters in depth approximately 50 meters offshore.

Distribution and bleaching in the field

A pilot study was done to determine the best substrate for collection. Snorkeling along the reef, samples were taken from coral rubble, dead coral heads, sand and the green alga *Halimeda* at depths between one and two meters. Samples from the dead coral heads were taken by scraping the surface into a plastic bag while the other substrates were taken whole in plastic bags. The coral rubble was placed in a colander that sat on a #100 sieve. The overhead seawater hoses in the wet laboratory were used to provide running water through the set up while the coral was scrubbed with a stiff brush to dislodge any organisms. The *Halimeda* was washed using the same technique. The collections obtained in the sieve, as well as the sand and coral head samples were placed in separate petri dish and examined under a microscope for 15 minutes each. Total abundance of *Peneroplis* from each substrate was used to determine the suitable habitat for collection.

Once coral rubble was chosen, the coastline was mapped by hand starting at the southern most accessible site. Starting at sea water outlet for the wet laboratory, the coastline was measured and markers were placed at three-meter intervals. Stopping at the northern property boundary gave a total of 81 meters of coast and 37 individual sites. The sites were assigned numbers on a map (0-9 were on the south reef, 10-37 on the north reef) and a random number table was used to select ten study sites for transects. A collection of coral rubble at each site was done at one, two and three meters of water depth. A PVC pipe cut to three meters and marked at one-meter intervals was used to measure water depth. Collections of coral rubble were made by snorkeling perpendicular to the shore at the marked site. As soon as the water depth obtained one, two, and three meters, a collection of rubble was taken. An initial temperature reading was also taken at each depth. One-gallon plastic bags were used in each collection and rubble was taken until the bags became full. All samples were washed for ten minutes using the previously describe design. After washing was completed, the sieve was shaken to obtain even distribution and the sample was divided into four pie shaped slices. A random number table was used to determine which slice was chosen and the sample was put in a petri dish. Using a 10x microscope, the sample was examined 15-minutes to find any *P. pertusus.* Each organism found was removed place in another dish to be categorized after the observation time had ended. The specimens were then looked at under 3x magnification to determine size and percent bleaching. Size was evaluated by measuring the test at its widest part and was

categorized as less than one mm (small), equal to one mm (medium) and greater than one mm (large). A few specimens were found to be two and three millimeters and were categorized as xlarge and xxlarge respectively. Bleaching was visually assessed through loss of purple pigment. This can typically be seen in two ways 1) color loss starting at the most recently added chamber and moving inward to varying degrees or 2) white spots appearing within the pigmented area. Percent bleaching was based on proportion of color loss in the whole organism and comparison to other specimens obtained.

To determine intrasample variability, a pilot study was done that compared abundance of *Peneroplis pertusus* in each of the four sample slices in the sieve. A pilot study into washing methods was completed to establish that a high enough proportion of the organism was obtained within ten minutes. This included two separate tests, done by washing the same rubble sample for ten minutes three consecutive times. To make a comparison with greater depths, a collection of rubble was made at nine meters by SCUBA divers certified in scientific diving by University of California Berkeley. Two one-gallon samples of rubble were taken, one each from the differing north and south sides of the reef.

Light manipulation study

Twenty individuals were gathered from coral rubble using previously described techniques. Each individual was placed in the plastic lid of small glass vials containing seawater. Each lid was numbered and the corresponding organism's degree of bleaching was recorded. The 20 organisms were randomly divided into two groups and placed in petri dishes also filled with seawater. One dish was placed directly under a full spectrum coral grow lamp (*get data for wattage and spectrum*) while the other was left on the laboratory countertop. At twenty-four hour intervals for three days, the organisms were observed under a 3x microscope. Percent bleaching was recorded.

Statistical Analysis

For the field study in distribution and bleaching, the percent bleaching data was turned into a proportion and a distributional analysis was preformed. Arcsine and square root transformations were preformed to try and obtain normal distribution. One way statistical analysis was used to compare site, depth, abundance and percent bleaching in individuals. The non-parametric Wilcoxon test was done on all pairs. If found to be significant in Wilcoxon, the Tukey-Kramer test was used to compare the differences in means. For the light manipulation study, proportion bleaching was averaged over the ten individuals in each the control and treatment group for each day. Means were and plotted over the four-day period for comparison in rate of increase.

Habitat description

A description of reef topography and composition was accomplished through visual observations while snorkeling. Notes were made with regards to sea floor, habitat distinctions, and rubble size.

RESULTS

Pilot studies

Collection from the various habitats yielded more *P. pertusus* from coral rubble than any other substrate (Table 1). When the four samples from the sieve were examined individually for abundance of *Peneroplis pertusus* they yielded nine, nine, eight, and nine individuals respectively. The two studies evaluating the ten-minute washing period are summarized in Table 2. The collection at nine meters depth yielded three individuals. Two

were found to be 100% bleached and the other was 25%.

Distribution and bleaching in the field

Statistically significant values, P<0.05 were found when comparing the differences of proportion bleaching by size, proportion of bleaching by site, and abundance of *P. pertusus* found at each site (Table 3).

Habitat	Number of Individuals Found
Halimeda sp.	0
Sand	1
Coral Head	0
Coral Rubble	11

Table 1. Number of *Peneroplis pertusus* collected from different substrates.

Wash	Study 1	Study 2
1	9	14
2	1	0
3	1	0

Table. 2. Number of individuals found after each wash. First study showing approximately 90% of all organism were obtained in the first wash, second study shows 100%.

The Tukey-Kramer results showed that bleaching in individuals was significantly different when comparing site ten to sites three and four. Site 10 had the highest average bleaching of 74% and sites 3 and four showed the lowest with 32% and 38% respectively. Differences in abundance by site showed no significance when using the Tukey-Kramer test. Bleaching by size analysis showed medium and small to be significantly less bleached than large but not differ from xl and xxl. Bleaching differed significantly with size of an individual but not with the depth at which it was collected. The number of individuals found in each size category is shown in Figure 1. Temperature readings taken at one, two and three meters did not vary and were found to be 28.6°C.

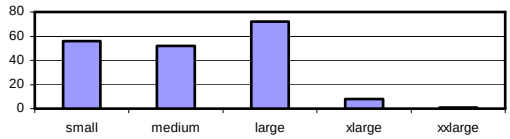

Figure 1. Number of individuals found in each size category showing similar results for small, medium, and large.

Light manipulation

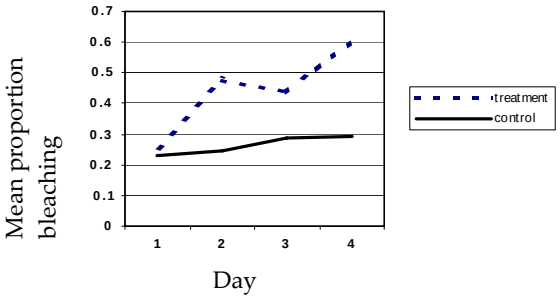

Figure 2. Mean proportion bleaching in treatment and control showing greater increase of bleaching in treatment group.

Light experiments showed a significant difference between the control and treatment group. Mean proportion bleaching increased at a much greater rate in the sample underneath constant photic stress (Figure 3).

One-way Analysis	P value
Depth by Size	0.1406
Bleaching by Size	<0.0001
Abundance by Depth	0.6217
Bleaching by Site	0.0021
Abundance by Site	0.0017
Bleaching by Depth	0.9748

Fig 3. Analyses performed and P values.

Habitat Description

Although the northern and southern sections of the fringing reef have different depth gradients, the substrate and samples collected from each had strong similarities. For both sections, the density of coral rubble covering the floor declined with an increase in depth. Filamentous algae cover on individual pieces of coral rubble appeared uniform throughout. The majority of rubble collected from one and two-meter depths had 100% of the upper facing surface covered in filamentous algae. Algae cover declined as water depth increased and samples from the three-meter collection typically had 50-100% cover. The northern reef had more interstitial sand due to a lower density of rubble. Although there was a difference in rubble density between the sides, the size variance between individual pieces appeared uniform throughout. Size of rubble collected from both sides ranged from $10cm^2$ to 216 cm^2 in top facing surface area. One major difference between the north and south sections of reef was the presence of the brown alga *Padina pavonea*. This alga has a distinctive fan shaped thallus and was present on approximately 75% of the coral rubble collected from the north reef at one and two-meters depth. *P. pavonea* was found to be completely absent in the south section of the reef. *Halimeda* used in collection was also found only on the shallow sloping north reef. It was found on larger coral rubble with a top surface area between $0.09m^2$ and $0.37m^2$ with a depth between 0.5 and 2 meters. No difference was detected in the size and number of larger coral heads between the sides.

DISCUSSION

Pilot studies

The study examining all samples of sediment within the sieve showed intra-sample variability to not be an issue. The washing study showed ten minutes to be a suitable time frame to obtain a high percentage of the study organism. Coral rubble was found to yield the highest abundance of *P. pertusus.* This is consistent with previous studies that found the organism to mainly inhabit coral rubble (Renema 2003). Hohenneger *et al* (1998) found various species Peneroplids including *P. pertusus* to be better adapted to coral rubble environments when compared to sand. The SCUBA collection at nine meters resulted in only a few specimens. This could be for a few reasons. Filamentous algae are the preferred habitat of Peneroplids in high-energy environments (Hoheneggar 2004). Having weak pseudopods, this habitat provides protection and a mechanism for attachment (Hohenneger 2004). Coral rubble collected from nine meters showed little (< 20%) to no algae cover. The sediment obtained from washing the rubble was much finer and possibly did not provide the protection or habitat composition desired. Light availability could be another factor. Nine meters depth might not provide the light penetration that *P. pertusus* is best suited. Further sampling at this depth would allow for greater comparisons.

Distribution and bleaching in the field

Abundance distribution did not vary with depth. An explanation could be that light penetration does not vary significantly at 1-3 meters. Light is an important limiting factor affecting distribution in larger foraminifera (Hohennegar *et al* 1998). Being host to photosynthetic symbionts restricts *P. pertusus* to the photic zone of the ocean. Logistics prevented light measurements from being taken at the varying depths in this study but Hohenegger *et al.* (1999) found *Peneroplis pertusus* to be highly adapted to extreme (80-100%) and very strong (60-80%) surface irradiance. This could also explain why bleaching was not found to be a factor with depth. The adaptation more intense light could serve as an indication of light availability at

depths. Population shifts towards greater depths could signal an increase in the solar radiation that reaches the ocean floor. Evidence for light induced bleaching in corals has been documented. The studies show that greater bleaching occurs on more exposed portions of coral than in areas lying under shadows of fixed objects (Williams *et al* 1990). It is possible that the increase in light also correlates to an increase in temperature.

Temperature variance between one and three meters was not detected in this study. This could be due to instrument sensitivity or the shallow depth gradient being investigated. Temperature typically decreases with depth and is a key factor limiting all symbiont-bearing foraminifera (Hohenegger 2004). Temperature influences the abundance of dissolved organic and inorganic particulate matter available to foraminifera (Hohenegger 2004). Being an ectoderm, temperature strongly affects the metabolic rate of *P. pertusus.* When temperatures are low, more dissolved nutrients are present but metabolism is slowed (Hohenegger 2004). Increased temperatures raise metabolic rates but lower the amount of dissolved nutrients. Temperature also controls the abundance of dissolved CO_2 in the ocean. Concentrations are low in warmer waters and this can hinder photosynthesis (Hohenegger 2004). The lack temperature variance could explain why abundance and bleaching in *P. pertusus* does not vary with depth.

There is a supposed temperature niche optimum of 28°C for foraminifera (Hohenegger 2004), which is close to temperature recorded in Mo'orea. This study supports this finding and suggests that *Peneroplis pertusus* is living under suitable temperatures for normal population dynamics. Little is known about the temperature niche of foraminifer's symbionts or their response to extreme highs and lows (Hohenegger 2004). Investigation into temperature tolerance in foraminifera would allow for better understanding how it effects the population and of its usefulness as a bioindicator in coral reefs.

Test size and abundance were not found to significantly vary by depth in this study except when looking at the very large individuals. The stable distribution of individuals found with small, medium and large test size shows level population distribution. This may be explained by even an even distribution in generations and steady reproductive dynamics (Hohenegger *et al* 1999). The years of highest bleaching incidences in *Amphistigina*, showed the lowest proportion of juveniles (Hallock et al 2006). Other studies found that severe bleaching in *Amphistegina* spp. showed reproductive failure (Williams *et al.* 2004). When partially bleached *Amphistegina* spp. Individuals attempted to reproduce asexually, they either produced hundreds of tiny cells that failed to calcify, or produced a few malformed offspring (Hallock et al 2006). Healthy unbleached specimens can produce hundreds of identical viable offspring. If *Peneroplis* spp. was experiencing these conditions, a skewed size distribution with preference towards larger individuals would be expected. The few individuals found in the largest size category are not suspected to a result of this and a larger sample size would help confirm this. If trends hold true for all symbiont bearing foraminifera, it suggests that *Peneroplis pertusus* is not experiencing stressors that effect natural reproduction. Bleaching found is thought to a natural occurrence of the population.

The strongest correlation in this study was found between bleaching in an individual and its test size. An explanation of this could be natural aging of the individual. Deterioration as the organism ages might lead to break down or loss of the endosymbiont. Another reason could be the demands of increased sized. As the organism grows, its energy needs increase possibly putting a strain on the symbiotic relationship. Studies in another larger symbiont bearing foraminifera *Amphistegina* showed that color loss was rarely seen in specimens smaller than 0.5mm but most specimens whose

diameter exceeded 0.8mm showed signs of bleaching (Hallock 2006). This study result suggests the same trends occurring in *Peneroplis pertusus.*

Light Manipulation

A significant difference between bleaching in the control and treatment group was found. This supports previous studies that found solar radiation to be a key factor bleaching in larger foraminifera. Studies on experimental bleaching of *Amphistegina gibbosa* in a laboratory setting showed a very limited optimum irradiance level (Williams *et al.* 2004). Talge *et al.* (2003) demonstrated that light induced bleaching seen in the laboratory showed similar physiological responses as bleaching in specimens collected in the field. Under prolonged photic stress, bleaching occurred even when under optimal temperatures (Talge *et al.* 2003). The connection between bleaching in the laboratory and bleaching in the field is an important link when applying experimental observations to specific habitats.

Habitat Description

The main difference between the two reefs was the depth gradient. Other factors include the presence of *Padina pavonea* and the slightly lower density of coral rubble on the north reef. It is unlikely that these factors alone contributed to the difference in proportion bleaching found between the specific sites: three, four, and ten. With no overall trend in bleaching between the two main reef sites it is difficult to know if habitat was actually a factor. A larger sample size would strengthen the correlation.

CONCLUSION

This study suggests that the abiotic stresses experienced between one and three-meter depths are not severe enough to alter population dynamics in *Peneroplis pertusus* of Mo'orea. Experimental evidence was found to support the correlation between increased solar radiation and bleaching in foraminifera. This can indicate *P. pertusus* usefulness as a bioindicator in the future. Comparison with similar studies in the future will allow for better understanding of changes in Mo'orea's population of *Peneroplis pertusus.*

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