Bleaching Patterns at varying Depths in the Family Fungiidae found in Moorea, French Polynesia
Alexis Howard, Lee Reeve, and Angela Johnson

Introduction

The term ‘coral bleaching’ refers to the loss of color in organisms that have a symbiotic relationship with dinoflagellate algae known as zooxanthellae. The loss of color is associated with the termination of this symbiotic relationship. Bleaching can be caused by disease, and abiotic factors such as changes in sedimentation, salinity, light and temperature. It has been suggested that global climate change is increasing the frequency of disease and causing abiotic changes, thus increasing the frequency of bleaching events (Fautin & Buddemeier 2004).

The Adaptive Bleaching Hypothesis (ABH) relies on the assumptions that (1) most zooxanthellae species can live in alternative hosts and that hosts can associate with multiple types of zooxanthellae, (2) different species of both hosts and zooxanthellae respond differently to varying abiotic conditions, (3) bleached individuals can reacquire zooxanthellae from their environment, and (4) the fitness of the host is affected by the zooxanthellae it associates with (Kinzie et al 2001, Fautin & Buddemeier 2004). Given these assumptions the ABH posits that in the event of an environmental change, species in which there is a symbiotic relationship with zooxanthellae expel zooxanthellae that are no longer beneficial, potentially reestablishing a relationship with a new symbiont (Kinzie et al 2001, Fautin & Buddemeier 2004).

We tested several of these key assumptions of the ABH using species from the family Fungiidae as model organisms. Fungiids are an excellent study species as they are free-living, and can be easily transplanted in the field and relocated to the lab. We tested three specific questions that relate to these assumptions: (1) If a Fungiid is taken from the depth at which it is living and moved either shallower or deeper, will it bleach? (2) If a Fungiid bleaches as a result of an abiotic change in its environment, will it reacquire different zooxanthellae? (3) If recently bleached Fungiids from an unnatural depth are relocated to their original depth, will they re-uptake zooxanthellae at that depth?
We hypothesize that (1) taking a fungiid from a depth at which it is living and moving it to a shallower or deeper depth will cause it to bleach. (2) After a Fungiid bleaches as a result of one or more abiotic changes in its environment, it will acquire new zooxanthellae from its new environment. (3) If a recently bleached Fungiid is moved back to its original depth, it will reacquire zooxanthellae from its original habitat.

Materials and Methods
Species Descriptions

Fungiidae are commonly known as free-living coral, however, their juvenile, or anthocaulus, stage is attached to the substrate. Fungiids are generally disc-shaped, but shape can vary from elongate forms to more circular morphs. Small teeth are located on the septa of the top portion of the Fungiid. These teeth can vary from very sharp to dull in texture. The underside of Fungiids contains rows of spines called costae. Species can be identified by differences in septa and costae structure. Because these differences are subtle, most species are difficult to identify in the field. Short tentacles can protrude out between the septa (Veron 1986 & 2000).

In this study, multiple species in the family Fungiidae were used. The common Fungia subgenera found in Opunohu Bay, Moorea, French Polynesia, are Ctenactis, Danafungia, Fungia, Pleuractis, and Verillofungia (Grant & Manning, unpublished). We also used the common Fungiidae species Herpolitha limax. H. limax differs from Fungia species in that it is colonial, while Fungia individuals contain a single polyp.

Site Description

Two arrays were set up at Coconut Grove, in West Opunohu Bay, between shore and the boat channel, along the face of a wall that drops to approximately 70ft. Water temperature at Coconut Grove ranged from 26.92°C at 5ft. to 26.83°C at both 20ft. and 40ft., and 26.81°C at 60ft.

One array was set up at West Opunohu, in West Opunohu Bay, between the boat channel and the crest. This site consists of a sandy slope that drops gradually from 5ft. to 50ft. Water temperatures at West Opunohu ranged from 26.78°C at 5ft., 27.2°C at night to 28.28°C during the day at 20ft., 27.2°C at night to 27.74°C during the day at 40ft., to
27.24°C at 60ft. There was a relative decrease in light intensity with increasing depth at both sites.

*Initial survey*

After the first few days of data collection at Coconut Grove, we noticed that most individuals at five feet were found in sheltered areas. When we moved them into exposed areas, they bleached just as readily as the Fungiids that were moved up from deeper depths. Once we noticed a pattern of shading compared to the amount of bleaching on an individual at Coconut Grove, we conducted observational transects. At 5, 10, and 20ft. depths, 30 meter transects were conducted parallel to the wall. The amount of shading, complete, partial, or exposed, was recorded along with the relative percent bleaching. Our results indicated that there was a relationship between shading and percent bleaching such that shaded individuals were less bleached. (Figure 1)

*Bleaching rates and intensity across species when individuals are transplanted to areas of different depths*

To test the idea that bleaching rate and intensity will vary across species when individuals are transplanted to areas of different depths, we did the following experiment. Multiple species from the family Fungiidae were transplanted across a depth gradient at three study sites, two on the wall at Coconut Grove and one on a sandy slope at West Opunohu. An array of flags was set up at each site on an initial transect line going from 5ft.-55ft. in depth at Coconut Grove (Coco1), and from 5ft.-50ft. in depth at West Opunohu (WestO). At the 5ft., 20ft., 40ft., and 50/55ft. depths additional transects were set up from the main transect line, following the depth contour. The second Coconut Grove site (Coco2), was set up along a main transect running from 10-50ft. in depth with depth contour transects at 10ft, 30ft, and 50ft. Two fungiids from each of the depths were placed every two meters along all the depth contour transect lines and flagged (Fig 2). Fungiids at West Opunohu were transplanted from Coconut Grove, as they are not found living at the sand flat in West Opunohu where the study was set up. However, we thought it was important to replicate the study on the sandy slope to see if there is
increased movement on a more level substrate. Degree of bleaching was measured every day or two for two weeks by taking digital photographs of each individual and overlaying a grid on the photo, allowing us to compare the total number of grid squares covering the Fungiid versus the number of squares covering bleached regions of the Fungiid. From this data we also calculated a bleaching rate (loss of colored tissue per day).

Reacquisition of zooxanthellae following bleaching at the transplant site

After relocating our individuals to the varying depths at the three sites, Coco1, Coco2, and WestO, we continued to monitor the Fungiids using photographs. We hoped to see an increase in pigment once the pigment had been lost all together. After taking a series of pictures for a few days after the bleaching had occurred, the photographs were processed and compared. Individuals that did not show bleaching to begin with were continually monitored as well.

Regaining zooxanthellae from an Individual’s original depth once bleaching has occurred at another depth

To determine whether these different species of Fungiids, once moved and bleached, would be able to restore their health by regaining zooxanthellae from their original depth, an experiment was performed. This experiment involved taking the Fungiids that had bleached in the previous experiments and moving them back to their original depth. After reintroducing them back into their original habitat, we observed them for several days by taking photographs. These photographs were then processed by placing a grid on the picture to figure out the percent bleaching of the individual.

Results

Through field studies at 3 different site locations, a pattern of bleaching due to depth variation was found. Overall, we found that individuals from the deeper depths at all three sites had the highest tolerance to abiotic changes. Another overall pattern of the data was that there was not a high correlation between percent bleached and day of experiment at any of the sites.
Bleaching rates and intensity across species when individuals are transplanted to areas of different depths

For the data collected from all three sites ANOVA tests were conducted using Systat. In this first experiment, at all three sites we found that the interaction between location and original depth had the most effect on bleaching. At Coco1 for the interaction between location and original depth, \( R^2 \)-value = 0.304, Degrees of Freedom = 9, and \( P = 0.000 \) (Fig 3). However, all variables, location, original depth, and day were significant. At Coco2 for the interaction between location and original depth, \( R^2 \)-value = 0.428, Degrees of Freedom = 4, and \( P = 0.001 \) (Fig 4). At this site, the effect of day alone did not turn out to be significant (Degrees of Freedom = 5, \( P = 0.102 \) (Fig 5). At our third site, WestO, for the interaction between location and original depth, \( R^2 \)-value = 0.322, Degrees of Freedom = 9, and \( P = 0.011 \) (Fig 6).

Reacquisition of zooxanthellae following bleaching at the transplant site

In this experiment, we did not perform any statistical analysis because of time constraints, although, from the raw data it looked unlikely that there was a pattern.

Regaining zooxanthellae from an Individuals original depth once bleaching has occurred at another depth

We were unable to collect enough data to perform statistical analysis for this experiment. A bar graph was made to show changes in percent bleaching between the first and last days of monitoring this experiment (a timespan of 4 days) for Coco1. The graph suggests that bleaching continued to increased in general(Fig.7). There seemed to be no trend at Coco2.

Discussion

Bleaching rates and intensity across species when individuals are transplanted to areas of different depths

At our first site at Coconut Grove (Coco1), our data showed that there was a definite relationship between the location in depth of the individual and the percent bleached (Fig.8). We had expected individuals to bleach at all depths besides their depth
They mainly reacted when we moved them up. Because there was not a very great difference in temperature between the deepest and shallowest depths, it seems likely that light levels were the main cause of bleaching. The same pattern was seen at our second Coconut Grove site (Coco2) (Fig. 9), except that the percentage of bleaching increased slightly at 50ft. which could be due to a data processing error. You may also assume that shallow zooxanthellae are unable to withstand the conditions at deeper depths since individuals moved up seem to be more resilient than species moved to deeper depths. The colonial species, *Herpolitha limax* is the most dominant species found at depths over 40 feet possibly, explaining why they are able to withstand change. In our experiments, we noticed that they seem to do much better than the *Fungia* species suggesting that they are a hardier species.

There also seemed to be no day effect at any of the sites, however there was a spike in bleaching around day 8. A possible explanation could be the change in the weather towards the end of our experiment. Increased swell causes the water column to be mixed, perhaps leading to the distribution of zooxanthellae; the zooxanthellae in symbiosis with the study individuals would also be able to better adapt to a mixed water column. There would also be less of a temperature difference in areas of high swell causing one less abiotic change for the fungiids to adapt to.

**Reacquisition of zooxanthellae following bleaching at the transplant site**

One of the assumptions of the Adaptive Bleaching Hypothesis is that bleached individuals can reacquire zooxanthellae from their environment. We hoped to test this assumption by causing Fungiids to bleach and then to observe them regaining zooxanthellae. However, we did not collect enough coherent data and did not have time to analyze the pictures that we took. Our method was essentially the same as for the first experiment, so this section could be repeated successfully. No patterns are obvious in our raw data, and we are unable to draw any conclusions from this experiment.

**Regaining zooxanthellae from an Individual’s original depth once bleaching has occurred at another depth**

An insufficient amount of data was collected and analyzed to show any real
trend for this experiment. One would need to continue this for an extended period of time. In addition, you would have to know whether or not the Fungiid was still alive after bleaching, which is extremely difficult because you would have to see the polyps out which is a rare occurrence.

The results of our first experiment show strong evidence for the validity of two assumptions of the ABH, namely that different species of both hosts and zooxanthellae respond differently to varying abiotic conditions and that the fitness of the host is affected by the zooxanthellae it associates with. Understanding the dynamics of the symbiosis between scleractinian corals and their zooxanthellae is critical to coral reef ecology and to reef conservation and management. It is widely believed that coral reefs are currently subject to bleaching events that are becoming frequent and intense (Fautin & Buddemeier 2004). Our results comply with previous evidence showing that light and/or temperature (we were unable to differentiate between the two in our study) increases cause corals to bleach, though we were unable to show conclusively that decreases in light and temperature also cause bleaching (Kinzie et al 2001). Our data also suggests that some zooxanthellae species are more resilient, or better adapted to living in a variety of conditions, than others. Our data analysis showed that Fungiids that were moved up from 60ft. and 40ft. were more resistant to bleaching than those that were moved down from shallower depths. One of the important assumptions we made when designing our experiment was that zooxanthellae are strongly stratified in the water column. Taking this assumption into account, our data implies that the deep zooxanthellae in Opunohu Bay are more resistant to bleaching than the shallow species. If nothing else, this means that there are zooxanthellae that are able to persist through increases in temperature and light intensity.

Another experiment we had originally planned on conducting was measuring Fungiid movement. It has been shown that Fungiids have excellent photoreception, and that they position themselves to maximize the efficiency of their zooxanthellae (Yamashiro and Nishihira 1995). We hoped to find out if transplanted Fungiids would try to move back toward their original depth rather than bleach right away. However, after a couple days of monitoring the Fungiids at Coconut Grove, it seemed clear that the
Fungiids were not moving, and if they had, it would be very difficult for us to quantify their movement. We set up the West Opunohu transect to account for this, as we thought that it would be an environment in which they would surely be able to move around, and it would be easy for us to observe their movement there. However, the majority of the individuals transplanted to West Opunohu were predated upon by the titan triggerfish, *Balistoides viridescens*, whose territory happened to occupy the space in which we set up our experiment. This predation caused the death of many Fungiids, and as the fish ate them they also moved them around and turned them upside-down. Fungiids were slowly disappearing and some would disappear altogether, which may have altered some of our data, causing Fungiids to bleach due to the stress levels of being eaten. In order to prevent this from occurring cages may have been used to protect them from predation.

There were several problems with the experimental methods and analyses we used. Data analysis would have been highly improved if we had a method to ensure the pictures were the same every time, for example angle, lighting and size. Use of the same camera with a strobe to ensure similar lighting would have also been useful, because some Fungiids would seem bleached when it was simply bad lighting. It may have been useful to have data collections more frequently and especially not skipping any days.

In the future, it would be interesting to perform some lab experiments to further prove or disprove our hypotheses. In order to test whether or not bleached Fungiids are capable of uptaking zooxanthellae from a different depth you may do a lab experiment with Instant Ocean, bleaching and switching Fungiids. To see if there is an explicit change in zooxanthellae, testing whether it is an adaptive change would be useful.

Some additional fieldwork can be done to test for shading preferences by transplanting corals to different shade levels and observing movement. Transects should also be done to show species differences, by setting up a transect consisting solely of *Herpolitha limax* and one of *Fungia simplex*, for example. This would show if the colonial species is in fact more robust than an individual consisting of a single polyp.

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Figure 1: Array design
Figure 2: Bleaching as a function of shading level

Figure 3. Percent bleached as a function of original depth x location at Coco1.
Least Squares Means

Figure 4. Percent bleached as a function of original depth x location at Coco2.

Least Squares Means

Figure 5. Percent bleached as a function of original depth x location at WestO.
Figure 6. Bleaching as a function of day at Coco2.

Figure 7. Percent change after bleaching
Figure 8. Percent bleached as a function of location at Coco1.

Figure 9. Percent bleached as a function of location at Coco2.
Figure 10. Percent bleached as a function of location at WestO.
References


