UNIVERSITY OF HAWAII AT MANOA BIOL 403: FIELD PROBLEMS IN MARINE BIOLOGY



Effects of Temperature, Salinity, pH, Reef Size, and *Tripneustes gratilla* on the distribution of *Montipora dilatata* in Kaneohe Bay

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Abstract:

The rare species of coral, *Montipora dilatata*, is considered a Species of Concern (SOC) by the National Oceanic Atmospheric Administration (NOAA) National Marine Fisheries Services (NMFS). It is a highly plastic coral with encrusting, plating, and branching morphologies, making it difficult to identify. The distribution of *M. dilatata* in Kaneohe Bay was studied by measuring temperature, salinity, pH, and reef size. *Montipora dilatata* colonies were found on seven of the twelve reefs surveyed. When a colony was found, temperature and water samples were taken, and colonies were marked with a GPS. Water samples were later analyzed for salinity and pH, and reef size was determined with ArcGIS. Data were analyzed with a one-way ANOVA for each variable with the presence or absence of *M. dilatata* on a reef. Analysis for temperature, salinity, and reef size, reefs with the occurrence of *M. dilatata* did not differ significantly different from reefs absent of *M. dilatata*. Results of this study may have future management implications for this Species of Concern.

The Hawaii State Department of Land and Natural Resources (DLNR) provided 1,000 native urchins, *Tripneustes gratilla*, as a biocontrol for the invasive red algae, *Eucheuma/ Kappaphycus*. Two weeks after urchin deployment, surveys found only four urchins in the vicinity of the release site.

Introduction:

Montipora dilatata is a rare species of coral with small populations in Kaneohe Bay

(Oahu) and the Northwestern Hawaiian Islands; it is considered a Species of Concern (SOC) by

the National Oceanic Atmospheric Administration (NOAA) National Marine Fisheries Services

(NMFS) (NOAA, 2007). Populations of *M. dilatata* have been on the decline due to

environmental stressors (Jokiel et al., 1983), especially thermal stressors (Jokiel & Brown,

2004). Jokiel and Brown (2004) found that M. dilatata was the first species coral to bleach in a

1996 bleaching event because of a temporary increase of 1°C in sea surface temperature of

Kaneohe Bay. Another coral stressor is changes in salinity that come from terrestrial freshwater

inputs (Faxneld et al., 2010; Williamson et al., 2011).

Kaneohe Bay was postulated to possess measurable gradients of salinity and temperature, attributable to freshwater inputs from Oahu and currents (Bathen, 1968). Additional studies have corroborated the sensitivity of corals, as well as other marine organisms, to fluctuations in

seawater pH (Caldeira *et al.*, 2007; Jokiel *et al.*, 2008). Ocean acidification, caused by increased concentrations of atmospheric CO₂, lowers saltwater pH and impedes the ability of corals to assimilate CaCO₃ to form their skeleton (Andersson *et al.*, 2009). Patch reef size can be another critical determinant of community composition, as larger reefs often contain more microhabitats and higher biodiversity than smaller ones (Huntington and Lirman, 2012).

The invasive red algae, *Eucheuma* spp. and *Kappaphycus* spp., both found in Kaneohe Bay, have spread rapidly and overgrown coral since their introduction in the 1970's (Conklin & Smith, 2005). One grazer known to significantly reduce the biomass of *Eucheuma* and *Kappaphycus* is the native urchin, *Tripneustes gratilla* (Figure 1) (Conklin & Smith, 2005). As a potential biocontrol, state of Hawaii Department of Land and Natural Resources (DLNR) released 1000 aquacultured individuals of *T. gratilla* onto Reef 44 in Kaneohe Bay in an attempt to decrease the biomass of *Eucheuma* and *Kappaphycus*.

In an effort to elucidate the distribution of *M. dilatata* across Kaneohe Bay, surveys were performed to measure physical and chemical parameters (salinity, pH, temperature, and patch reef area) at multiple reefs. In last year's BIOL 403 class, *M. dilatata* was found on reefs 44, 47, and 54 (Figure 2). If temperature, salinity and pH affect coral health and ecology, we would expect *M. dilatata* to be present on reefs similar to reefs 44, 47, and 54 with similar temperature, salinity, and pH. Since larger reefs contain more biodiversity, we would expect *M. dilatata* to be present on larger reefs. Because it is a Species of Concern, monitoring pH, thermal, salinity and habitat size of *M. dilatata* may provide information for future management of this species.

Materials and Methods:

Our null hypothesis was that there is no significant difference in temperature, salinity, pH, or reef size among reefs that affects the distribution of *M. dilatata* in Kaneohe Bay.

In 2011 the UHM class of BIOL 403 reported having confidently identified only 9 colonies of Montipora dilatata at reefs 44, 47, and 54. In 2012, we compared patch reefs hosting *M. dilatata* to those lacking the rare scleractinian; students surveyed one chemical variable and three physical parameters at multiple reefs across the Kaneohe Bay. Snorkelers scoured various reefs in search of *M. dilatata*; the criteria via which this polymorphic glabro-favoleate was identified were the following: an encrusting (Figure 3A), plating (Figure 3B), or columnar (Figure 3C) coral colony with a color spectrum ranging from chocolate brown to a light or even vivid purple hues for which the tips of the branches end in a smooth flattened surface deprived of verrucae or papillae. Reefs 44, 47, and 54 were presumed to possess parameters facilitating the proliferation of *M. dilatata*. Based on 2011 data (as well as personal accounts) reefs 11, 43, and 51 were noted as reefs that did not harbor *M. dilatata*. The investigation extended to reefs 12, 19, 20, 22, and 23 for a total of twelve surveyed reefs. As students came across new colonies, they would photograph them with an Olympus Stylus *Tough* (for later confirmation by coral specialists Dr. Hunter and Dr. Forsman), and mark their coordinates with the use of a Garmin etrex GPS.

When a potential *M. dilatata* colony was found, the temperature of the surrounding water was recorded and two water samples were taken with 50ml vials (Figure 4A). Temperature was measured by holding two thermometers next to the colony for twenty seconds (Figure 4B). Position of each colony was marked as a waypoint using a GPS and mapped through the use of ArcGIS software. Water samples were acquired by lowering the vials to the base of the potential *M. dilatata* colony and uncapping them, as to only capture the water directly next to the coral. Water samples were analyzed in the lab to measure salinity and pH. Seawater pH in each vial was measured with a Fisher Scientific AB 15 pH meter (Figure 5B). A Kahl Scientific Instrument refractometer (Figure 5B) was used to measure the salinity of the seawater in each vial. The area of each individual patch reef was estimated using ArcGIS software.

Alien algae control project

In association with DAR, 1000 juvenile *Tripneustes gratilla* were provided to the class of 403. Students decided to deploy the echinoderms across reef 44 as biocontrol agents to cull the overgrowth of *Kappaphycus spp*. mats occurring on the southern side of the reef. In an effort to protect the *M. dilatata* colony number 1 from potential overgrowth, approximately 200 *T. gratilla* were placed on the invasive algae around as well as on the colony. The remaining 800 were strewn about haphazardly wherever the alien algae were observed to aggregate. After two weeks, two surveys of reef 44 were conducted to survey the abundance of urchins.



Figure 1. A) Native urchin *Tripneustes gratilla* and B) invasive red algae *Euchema/Kappaphycus*.



Figure 2. Reefs 54, 47, and 44 were surveyed in a previous BIOL 403 class in summer 2011 for *M. dilatata* colonies.



Figure 3. A) Encrusting, B) Plating and C) Columnar morphology of *M. dilatata*.



Figure 4. A) Water samples and B) Temperatures were taken at each *M. dilatata* colony.



Figure 5. A) Fisher Scientific AB 15 pH meter and B) Kahl Scientific Instrument Refractometer instruments were used to measure pH and salinity (respectively).

Results

In Kaneohe Bay, the following reefs were surveyed for the presence of *M. dilatata*: 54, 51, 47, 44, 43, 24, 23, 22, 20, 19, 12 and 11 (Figure 6). At these reefs, temperature, salinity, pH and reef size were measured (Table 1). Of these 12 reefs, 30 colonies of *M. dilatata* were found on reefs 54, 51, 47, 44, 43, 12, and 11 and GPS points were recorded at each colony (Table 2). These GPS points were used to generate an ArcGIS map of the locations of *M. dilatata* (Figure 7).

The average temperature at reefs with the presence of *M. dilatata* was $26.57\pm0.45^{\circ}$ C. At reefs in the absence of *M. dilatata*, the average temperature was $24.90\pm0.22^{\circ}$ C. A one-way ANOVA was used to test the relationship between temperature and the occurrence of *M. dilatata* among the surveyed reefs. Differences in temperature were significantly different among reefs with the presence and absence of *M. dilatata* ($F_{(1, 18)} = 121.26$, P<0.05) (Table 3), so we rejected the null hypothesis H_{o1} . This was further demonstrated by the spatial distribution in Figure 8; reefs with the occurrence of *M. dilatata* have a higher temperature range than reefs in the absence of *M. dilatata*.

The average salinity at reefs with the presence of *M. dilatata* was 35.00 ± 0.40 ‰. At reefs in the absence of *M. dilatata*, the average salinity was 36.10 ± 0.65 ‰. A one-way ANOVA was used to test the relationship between salinity and the occurrence of *M. dilatata* among the surveyed reefs. Salinities were significantly different among reefs with and without *M. dilatata* ($F_{(1, 18)} = 15.61$, P<0.05) (Table 4), so we rejected the null hypothesis.. This was further demonstrated by the spatial distribution in Figure 9; reefs with *M. dilatata* had a lower overall average salinity than reefs without *M. dilatata*.

The average pH at reefs with *M. dilatata* was 8.13 ± 0.03 . At reefs where *M. dilatata* was absent, the average pH was 8.12 ± 0.02 . A one-way ANOVA was used to test the relationship between pH and the occurrence of *M. dilatata* among the surveyed reefs. The pH was not significantly different among reefs with the presence and absence of *M. dilatata* ($F_{(1, 18)} = 0.10$, P>0.05) (Table 5), so we failed to reject the null hypothesis.

The average reef size at reefs with *M. dilatata* was 41,690 m². At reefs in the absence of *M. dilatata*, the average reef size was 3,876 m². A one-way ANOVA was used to test the relationship between reef size and the occurrence of *M. dilatata* among the surveyed reefs. Reef size was significantly different among reefs with and without *M. dilatata* ($F_{(1, 10)} = 8.98$, P<0.05) (Table 6), so we reject the null hypothesis.

The morphology of *M. dilatata* varied among reefs so pictures of *M. dilatata* were taken for later analysis (Figure 10). Colony 20 on reef 43 was a potential *M. dilatata* colony with indistinguishable morphologies (Figure 11). That colony had characteristics of *Montipora capitata* with flattened tops, which are characteristic of *M. dilatata*.

Two surveys were conducted on Reef 44 at seven and fourteen days after deployment of *T. gratilla* as a biocontrol of *Eucheuma/Kappaphycus*. These surveys yielded a total of four urchin sightings across the entire reef. During surveys to find *Eucheuma/Kappaphycus*, it was discovered that the invasive algae was present on both the northern most and southern most Colonies (1 & 3) but not on Colony 2.

Reef	Salinity	Temperature				
Number	(‰)	(°C)	pН	Area (m2)	Occurrence	
54	35.00	27.00	8.125	87268.392	Presence	
51	34.67	26.50	8.127	68519.023	Presence	
47	35.00	27.00	8.185	38145.137	Presence	
44	35.33	27.00	8.107	45024.144	Presence	
43	35.20	26.00	8.158	21268.319	Presence	
12	35.50	26.00	8.080	13077.378	Presence	
11	34.33	26.50	8.143	18504.206	Presence	
24	35.00	24.50	8.095	9944.588	Absence	
23	36.50	25.00	8.120	3281.193	Absence	
22	36.50	25.00	8.110	1812.698	Absence	
20	36.00	25.00	8.160	2465.751	Absence	
19	36.50	25.00	8.135	1874.08	Absence	

 Table 1. Average physical and chemical parameters of each surveyed reef and the occurrence of *M. dilatata*.

Reef	Colony	Latitude	Longitude	
Number	Number	(degrees)	(degrees)	Status
54	7	21.49150	-157.83658	Confirmed
54	8	21.49160	-157.83672	Confirmed
54	9	21.49130	-157.83727	Confirmed
51	23	21.49294	-157.82951	Confirmed
51	24	21.49298	-157.82944	Confirmed
51	25	21.49317	-157.82957	Confirmed
47	4	21.48090	-157.83267	Confirmed
47	5	21.48170	-157.83289	Confirmed
47	6	21.4811	-157.83339	Confirmed
47	10	21.4817	-157.83301	Potential
47	11	21.4817	-157.83301	Confirmed
47	12	21.4817	-157.83301	Confirmed
47	13	21.4817	-157.83301	Confirmed
47	14	21.4817	-157.83301	Confirmed
47	15	21.4817	-157.83301	Confirmed
47	16	21.4806	-157.83322	Confirmed
47	17	21.4812	-157.83353	Confirmed
44	1	21.4771	-157.83173	Confirmed
44	2	21.4774	-157.83199	Confirmed
44	3	21.4777	-157.83220	Confirmed
43	18	21.47729	-157.82733	Potential
43	19	21.47750	-157.82693	Confirmed
43	20	21.47762	-157.82706	Potential
43	21	21.47755	-157.82674	Confirmed
43	27	21.47746	-157.82727	Confirmed
12	28	21.45060	-157.79803	Confirmed
12	29	21.45060	-157.79805	Confirmed
12	30	21.4507	-157.79802	Potential
11	22	21.44970	-157.79526	Confirmed
11	26	21.4493	-157.79578	Potential

Table 2: Reefs with confirmed colonies of *M. dilatata* and their GPS coordinates.

Table 3. One-way ANOVA showing Temperature versus Occurrence of	
M. dilatata.	

Source	DF	SS	MS	F	Р
Occurrence	1	12.80	12.80	121.26	0.00
Error	18	1.90	0.106		
Total	19	14.70			

Source	DF	SS	MS	F	Р
Occurrence	1	7.001	7.001	15.61	0.001
Error	18	8.072	0.448		
Total	19	15.073			

Table 4. One-way ANOVA showing Salinity (‰) versus Occurrence of *M. dilatata*.

Table 5. One-way ANOVA showing pH versus Occurrence of *M. dilatata*.

Source	DF	SS	MS	F	Р
Occurrence	1	0.00013	0.00013	0.10	0.751
Error	18	0.02282	0.00127		
Total	19	0.02295			

Table 6. One-way ANOVA showing Reef Area versus Occurrence of *M. dilatata*.

Source	DF	SS	MS	F	Р
Occurrence	1	4169874750	4169874750	8.98	0.013
Error	10	4641612276	464161228		
Total	11	8811487026			



Figure 6. Reefs that were surveyed for *M. dilatata* colonies in June 2012.



Figure 7. Montipora dilatata colonies found across Reefs 54, 47, 44, 43, 12 and 11 in Kaneohe Bay.



Figure 8: Average temperature (°C) at surveyed reefs in Kaneohe Bay. Red boxes indicate reefs with the occurrence of *M. dilatata*. Purple boxes indicate reefs with the absence of *M. dilatata*.



Figure 9: Average salinity (‰) at surveyed reefs in Kaneohe Bay. Red boxes indicate reefs with the occurrence of *M. dilatata*. Purple boxes indicate reefs with the absence of *M. dilatata*.



Figure 10. *Montipora dilatata* colonies from A) Reef 54, B) Reef 51, C) Reef 47, D) Reef 44, E) Reef 43, F) Reef 12 and G) Reef 11.



Figure 11: Colony 20, a potential *M. dilatata* colony on reef 43 with morphological plasticity. Appearance of *Montipora capitata* with flattened purple tops, characteristic of *M. dilatata*.

Discussion:

Students visually surveyed reefs 11, 12, 19, 20, 22, 23, 24, 43, 44, 47, 51, and 54 during the class of Biol 403 in 2012 to document the occurrence of *Montipora dilatata* colonies. In an effort understand relationships between seawater parameters on the reefs at which the UHM class of Biol 403 confidently identified colonies of *M. dilatata* in 2011 and the actual incidence of the rare montiporid, the salinity, temperature, size, and pH of the patch reefs were computed and digitized using ArcGIS. As students inspected a dozen reefs and analyzed countless coral colonies, the group would scrutinize each potential *M. dilatata* located by a student until a consensus was reached.

The inherent difficulty associated with the visual identification of morphologically plastic corals extends even to the genetic field as mitochondrial markers lack the resolution to differentiate relatively recent mutational divergences within congeners (Forsman et al. 2009). For this reason, it was imperative that the group agreed on the criteria by which *M. dilatata* would be identified. However, some congeners such as, *Montipora turgescens*, and *Montipora flabellata* have been documented to share a deceptive amount of physiological overlap and displaying no perceptible genetic differences (Forsman et al. 2010). Certain confounding specimens, such as colony 20 from reef 43, resembled hybrid species between *M. dilatata* and *M.*

capitata and were photographed for confirmation by coral experts. Our group would consider any coral morphology as *M. dilatata* as long as its colors ranged between purple to chocolate brown and it possessed a branch with a smooth flattened lavender tops. *Montipora dilatata* was found on reefs 11, 12, 43, 44, 47, 51, and 54. Surveyed reefs 19, 20, 22, 23, and 24, which were devoid of *M. dilatata*, were used as control reefs.

Out of the four parameters to which *M. dilatata* occurrence was compared only three returned statistical significance through ANOVA testing. The temperature gradient measured across the bay revealed consistently colder water at the at the patch reefs lacking colonies of *M. dilatata*. Although most studies for thermal stress on the genus *Montipora* have been done documenting the effects of elevated water temperature (Coles and Jokiel 1977; Dove and Ortiz 2006; Jokiel and Brown 2004). Yet our data would suggest that *M. dilatata* faces a low temperature threshold within Kaneohe Bay. Reefs documented, by the Biol 403 class of 2011 as having the largest *M. dilatata* colonies were documented to have the highest temperature of all the reefs surveyed in 2012. As much as a 2.5°C disparity was observed between reefs with established *M. dilatata* colonies and the appreciably cooler reefs without the target hermatypic coral. It is interesting to notice how the thermal gradient documented by the Biol 403 class of 2012 agrees with past research by Bathen (1968).

Another parameter that was deemed significant (ANOVA test), was the incidence of *M*. *dilatata* compared to seawater salinity across the twelve surveyed patch reefs in the bay. The control reefs (19, 20, 22, 23, and 24) had a significantly higher salinity than those hosting the scleractinian coral. This finding is counterintuitive to most research, which correlates proximity to near shore freshwater effluent as producing coral stressors engendered by low salinity (Faxneld et al. 2010; Williamson et al. 2011). Our data suggests that higher salinity can be just

as significant of a stressor as low salinity, and a strong determinant of *M. dilatata* distribution in Kaneohe Bay. Perhaps the lower salinities found in the north of Kaneohe Bay can be attributed to three streams (Kahaluu, Kaalaea, and Haiamoa), which enter the Bay near reef 44.

The last parameter that was significantly tested with ANOVA was the size of individual patch reefs. After examining numerous reefs, it was quickly brought to the students' attention that the occurrence of *M. dilatata* may be size dependent, with regards to the area of each patch reef. Reefs 11, 12, 43, 44, 47, 51, and 54 were characterized as larger reefs (mean area of 41,690m²), hosted *M. dilatata*. Reefs without *M. dilatata* were considerably smaller (mean area of 3,876m²). A broadcast spawner, such as *M. dilatata*, may simply be less frequent on smaller reefs because they provide smaller targets for larvae to fortuitously come across and settle on. Larger patch reefs seem to be a preferred habitat of *M. dilatata* as more space may be associated with less competition. A patch reef with a higher surface area may provide a higher frequency of microhabitats suitable for *M. dilatata* larvae settlement, successful metamorphosis and development.

Seawater pH was not determined to fluctuate in any appreciable amount, in relation to the incidence of *M. dilatata* colonies, according to ANOVA testing. However the bay's pH has been known to fluctuate quite a bit on a daily basis, which is likely to grant the corals residing in the bay a higher tolerance to pH fluctuations. The pH in the bay would appear to be within a range that is conducive to *M. dilatata* growth in every area that was surveyed. Although we did not expect to see any large variations in pH across Kaneohe Bay, we hypothesized that this data would provide insight for future studies on environmental change.

As far as temperature and pH in Kaneohe Bay, we were not anticipating any notable patterns. We found that the data collected would provide future studies of climate change and

ocean acidification with a good baseline of information to work from, and perhaps even give future Biol 403 classes a source to which they could compare their data in the event that anthropogenic changes alter the physical and chemical parameters of Kaneohe Bay.

The 1000 juvenile *T. gratilla* that were released on reef 44, to cull the growth of *Kappaphycus spp.*, were monitored on four separate occasions for two weeks after their initial deployment. Only 4 out of the 1000 echinoderms were found by pulling up mats of *Kappaphycus spp.*, which they appeared to be successfully including into their diet, during these surveys. However, we suspect that many more still reside on the reef but are hiding in the mats of *Kappaphycus spp.* as well as the very rugose topography of reef 44. These urchins' behavior has been documented, and it would appear normal for juvenile *T. gratilla* to seek shelter in the reef to reduce the amount of harassment inflicted by wrasses while they are still juveniles (Dafni and Tobo, 1987).

When it comes to measuring physical and chemical parameters in a marine environment, there are other factors to consider. In this study, there were restrictions on surveying time that caused data collecting to be spanned out over three weeks. Throughout the surveys, the weather was variable in the fact that some days it was raining and others it was not. Also, using mercury thermometers to determine the temperature in a reef is not as accurate as scientists would like. In future studies, HOBO data loggers or other type of thermal data logger, where a long-term study of the reef can be performed would be recommended.

Further research that should be conducted would be to 1) expand collections to current and sedimentation studies, 2) survey reefs over a longer duration of time, 3) expand surveys to multiple reefs, 4) attempt to determine whether *M. flabellata* and other *Montipora* species can morph into *M. dilatata*, and 4) expand survey times to other months of the year. These future

research studies can aid in management of this genus and species and the identification through the knowledge of its morphological plasticity.

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