Field surveys for two National Marine Fisheries Service Species of Concern, Lingula reevii and Montipora dilatata, in Kane'ohe Bay, Oahu, Hawaii.



Unique siphon openings formed by Lingula reevii burrows.

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#### **Background and Problem Statement**

The National Marine Fisheries Service (NMFS) defines a Species of Concern (SOC) as a species that is not being considered actively for listing under the Endangered Species Act (ESA) but for which significant concerns or uncertainties regarding its biological status and/or threats exist (69 FR 19975). The purpose of the NMFS SOC Program is to conduct proactive conservation activities to preclude the listing of future species, rather than addressing the recovery needs of a species listed. Two of the Pacific Islands Region Species of Concern are the inarticulated brachiopod, Lingula reevii, and the Hawaiian reef coral, Montipora dilatata.

There are twelve extant species of Lingula and most are limited to tropical and subtropical waters of the Indo-West Pacific (Hyman 1959). L. reevii is a filter feeding inarticulated brachiopod that can be identified by three small holes in the sediment, formed when the animal extends its lophophore with three siphon holes for feeding and respiration (see report cover photo). L. reevii was recently identified as a "species of concern" (Federal Register, 2004). This species is considered to be at risk due to habitat degradation and alteration, over-exploitation, pollution, sedimentation, vulnerable life history, and/or limited distribution (NOAA, 2007).

Lingula reevii has three recorded occurrences: 1) Kane'ohe Bay, Oahu, Hawaii; 2) Ambon, Indonesia; and 3) Japan. There are no data on the relative abundance of this species in Indonesia or Japan, nor has it been yet confirmed by genetic analyses that these three populations are all representatives of the same species. Past surveys of Kane'ohe Bay populations (Worcester, 1969; Emig, 1981) found a distinct decrease in abundance following the diversion of sewage effluent from the Bay in 1978/1979. In 2004, three University of Hawaii-Manoa (UHM) students and Dr. Cynthia Hunter conducted visual surveys in areas of historical L. reevii abundance as well as in areas appearing to have suitable habitat. Approximately 2,950  $m^2$  were surveyed and a maximum density of four L. reevii/ $m^2$  was observed, a precipitous decrease from previous maximum estimates of 100 individuals/ $m^2$  in 1981 and 500 individuals/m<sup>2</sup> in 1969. This decline in abundance of L. reevii in Kane'ohe Bay may be due to decreased organic enrichment from the sewage discharge diversion that occurred almost three decades ago, as well as the more recent reduction of suitable habitat by the invasion of mat-forming alien algae species. In addition to these factors, L. reevii is sessile and reproduces by broadcast spawning. Thus, individuals must maintain a minimal density to ensure successful fertilization of gametes. Therefore, the population status of L. reevii f in Kane'ohe Bay is considered to be at risk.

Montipora dilatata has been recorded from: 1) the main Hawaiian archipelago in Kane'ohe Bay, Oahu; and 2) the Northwestern Hawaiian Islands (Fenner, 2005; Maragos et al., 2005). In 2000, surveys of M. dilatata identified only three colonies in Kane'ohe Bay, where it formerly was more abundant (NOAA, 2007). Habitat degradation as a result of

sedimentation, pollution, alien/invasive algae species, and its historically limited distribution may be contributing factors to the apparent decline of this species in Kane'ohe Bay.

## **Goals and Objectives**

The goal of this study was to conduct extensive surveys of all suitable habitats for Lingula reevii and Montipora dilatata in Kane'ohe Bay, Oahu. To accomplish this goal, NMFS Pacific Islands Regional Office (PIRO) contracted Dr. Hunter to direct eight students in a University of Hawai-Manoa five-week summer course entitled "BIOL 403: Field Problems in Marine Biology". In this course, students are taught a variety of skills to conduct and analyze field research in marine habitats.

## <u>Specific Tasks</u>

The following tasks were performed:

- 1. Extensive surveys of all suitable habitats in Kane'ohe Bay were conducted in May/June, 2007, for the presence, absence, and relative abundance of Lingula reevii and Montipora dilatata;
- 2. The distribution and abundance of Lingula reevii were mapped; field identification of Montipora dilatata was confounded by overlapping characteristics with other Montipora species and thus its distribution and abundance could not be determined via field surveys.
- 3. The current population size was estimated for Lingula reevii but could not be determined for Montipora dilatata;
- 4. The occurrence of alien/invasive algae was quantified; and
- **5.** Habitat types, as well as substratum type, sand depth, sand grain size, and cooccurrence of Lingula reevii with other benthic organisms were characterized and photodocumented to improve understanding of the habitat requirements of this species.

## Summary of Results:

Extensive surveys of Lingula reevii and Montipora dilatata on fringing reef, patch reef, and barrier reef habitats within Kane'ohe Bay were conducted between May 16<sup>th</sup> and June 13<sup>th</sup>, 2007. Surveys were performed by eight students under the supervision of two instructors (Dr. Cynthia Hunter and Dr. David Strang) and a teaching assistant (Carrie Pederson), as part of University of Hawaii-Manoa's Field Problems in Marine Biology (Biol 403) 2007 course. Methods entailed GPS-rectified presence/absence surveys as well as quantitative transect surveys, habitat characterizations, sediment depth and grain size analyses, water depth, enumeration of co-occurring benthic organisms (including alien/invasive algae), and an analysis of L. reevii abundance in relation to recreational use of potentially environmentally-sensitive sites in Kane'ohe Bay.

Maps and tables of the distributions of L. reevii are provided in this report. Its abundance has further declined since this species was last surveyed (from a maximum

density of 4/m<sup>2</sup> in 2004 to 0.9/m<sup>2</sup> in 2007), but there was evidence of small size classes indicating recent recruitment within this population. There appears to have been a downward shift in maximal abundance with water depth, from 0.5 m (Worcester, 1969) to 0.6-3.8 m in the current study. L. reevii was most abundant in areas with a dominant sand grain size of 500 um, but there appeared to be no correlation with sand depth. L. reevii abundance was inversely correlated with the presence of other invertebrates, reef fish, and alien invasive algae. Recreational use of prime habitat for L. reevii (Sandbar) may also have negative impact on its abundance in this area.

Because of its similarity to a suite of congeners in Kane'ohe Bay, it was not possible to positively identify and characterize the abundance of the coral, Montipora dilatata in these surveys. Further morphological and genetic studies will be necessary to reliably estimate the numbers of this species in future field surveys.

## I. Abundance and Distribution of Lingula reevii in Kane'ohe Bay, Oahu

#### Introduction

*Lingula reevii* is an inarticulated brachiopod, or lamp shell. This filter-feeding organism is enclosed in two valves held together by muscle (rather than interlocking teeth and sockets as articulated brachiopods) and is attached to the substratum by an elongate pedicle. Adults can grow up to ~4 cm in valve length and the pliant pedicle may extend to over 20 cm (Emig 1978). The valves are a brilliant blue-green or emerald color and occasionally show orange or black toward the centers. Light-colored setae protrude from the frontal edges of the valves. This species burrows vertically in soft sediments, creating a uniquely recognizable three-holed siphonal opening, formed by the valve setae (see cover photo).

Past surveys have shown a significant decrease in population size of *Lingula reevii* from 1969 to the present in Kane'ohe Bay, where its previous maximum abundance was reported in earlier surveys as 500/m<sup>2</sup> (Worcester 1969), 100/m<sup>2</sup> (Emig 1981), and 4/m<sup>2</sup> in 2004 (Biol 403 students; Hunter, et al. in review). Past studies found that *L. reevii* was in highest abundance in the South Bay, with some individuals found on the Sandbar in the Mid Bay (Figure 1). Speculations on why such a decrease in its population size has occurred include decreased enrichment after sewage discharge was diverted from the Bay in the late 1970's, the recent increase in alien invasive algae, an increase in recreational activity in the bay (particularly at the Sandbar), and/or other unknown influences.

The purpose of this study was to gain information on current *Lingula reevii* abundance and distribution in Kane'ohe Bay, Oahu, along with concomitant habitat

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characteristics and anthropogenic activities in Kane'ohe Bay in order to make recommendations for conservation actions and future research needs.

The specific tasks of the current study included: 1) conducting qualitative and quantitative surveys of Kane'ohe Bay to map the current location and abundance of the *L*. *reevii* population, 2) characterizing and photodocumenting areas where *L. reevii* were found in terms of substratum type, water depth, sediment depth, sediment grain size, co-occurrence of other benthic organisms (including alien/invasive algae), and in relationship to nearby recreational activities. Each aspect of these analyses was conducted by individual students in the field course, and are presented below in Sections A-G.

#### A. Distribution and Abundance Surveys

#### **Materials and Methods**

The distribution and abundance of *Lingula reevii* was determined through extensive snorkeling surveys at 71 sites on the patch reefs, fringing reefs, dredged reefs, and Sandbar in Kane'ohe Bay from May 16, 2007 to June 13, 2007 (Figure 2). The presence of *L. reevii* was determined by counting the number of burrows in the substratum identified by their distinct three-siphon holes. Care was taken to not disturb the area prior to surveys. After a disturbance causing retraction of animals into the substratum (often just the movement or shadow cast by a snorkeler), re-establishment of the siphon holes at the sediment surface was observed to take up to six minutes.

Preliminary surveys were conducted to determine the presence or absence of *L. reevii* within a specific location. A total of 71 locations were surveyed and GPS latitude and longitude data were recorded using Garmin Geko 201 units for each location. In areas of

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higher abundance (>20 individuals found by 8 snorkelers within a 20 minute preliminary survey), quantitative transects were conducted covering a total area of 2,900 m<sup>2</sup>.

A "DACOR" method was used to qualitatively describe *L. reevii* abundance (Dominant = 401+; Abundant = 251-400; Common = 101 – 250; Occasional = 21 – 100; Rare 1- 20). DACOR criteria were based on previous studies in Kane'ohe Bay that reported a maximum *L. reevii* abundance of 500/m<sup>2</sup> (Worcester 1969).

A total of 24 DACOR transects in seven locations were conducted based on the results from the preliminary surveys where abundance was confirmed to be  $\geq$  Rare. The locations of the DACOR transects (Figure 1) were: Sandbar (SB) 2, Sandbar 3, Sandbar 4, Sandbar 6, Fringing Reef (FR) A, Dredged/Fringing Reef (DFR) H, and Dredged/Patch Reef (DPR) 0. Transects (30-60 m in length) were run in parallel (except for SB 6), separated by 5-10 m depending on the size, depth, and topography of the area surveyed (Figures 2-6). Pairs of snorkelers counted the number of *L. reevii* at every meter along each side of the transect line. Sediment and water depth were recorded every 10 m along the transects.



Figure 1. Kane'ohe Bay showing North Bay, Mid Bay, and South Bay delineations and locations where DACOR transects were conducted. Numbers are reef designations from Roy (1970); letters indicate locations of 2004 survey sites (Hunter et al. in review).





Figure 3. Layout of transects conducted at Sandbar 3, Sandbar 4, and Fringing Reef A sites.



Figure 4. Layout of transects conducted at Dredged Reef 0 site.

Om	Or. DPR O			
	50m			
60m				
Total Area = 220m2				

Figure 5. Layout of transects conducted at Dredged Reef H site.

Area =
120m2

Figure 6. Layout of transects conducted at Sandbar 6 site.



#### Results

The occurrence of *Lingula reevii* was very patchy, with highest overall abundance at the Sandbar (Figure 7). *L. reevii* was generally in low abundance on patch reefs (1-3 individuals observed per reef), with the exception of Reef 15, where 52 individuals were found within a small sandy area (Figure 8). At locations where quantitative transects were conducted, the highest average abundance of *L. reevii* (0.9 individuals/m<sup>2</sup> was found on

deeper, dredged reefs (Figure 9). The relative abundance of *L. reevii* per reef type is shown in Figure 10.

When comparing the North, Mid, and South Bay sectors of Kane'ohe Bay, the South Bay was found to have the highest abundance in terms of *Lingula reevii* per m<sup>2</sup> (Figures 11, 12). The highest total abundance per sector was in the Mid Bay, dominated by the sandbar habitat (Figure 13).



Figure 7. Lingula reevii abundance in Kane'ohe Bay, Oahu, Hawaii in May-June, 2007.



Figure 8. Total number of *L*. *reevii* found in quantitative surveys within each reef type.



Figure 9. Number of *L. reevii* per  $m^2$  found in quantitative surveys within each habitat type.

Figure 10. Total number of L. reevii divided by total area of reef type giving the abundance of L. reevii per  $m^2$  throughout habitat types in Kaneohe Bay.



Figure 11. Total number of *L. reevii* found in area surveyed for each section of the bay.

Figure 12. Number of *L. reevii* per  $m^2$  for each section of Kaneohe Bay.

Figure 13. Total number *of L. reevii* divided by total area of available habitat (<8 m depth) in Kaneohe Bay sectors.

#### Introduction

In summer, 2004, visual surveys were conducted by UH Biology 403 students throughout Kane'ohe Bay to determine the abundance and distribution of *L. reevii*, in areas where they were historically found and in areas that appeared to have suitable benthic habitat (i.e., mostly shallow 1-3 m depths on sandy reef flats). 107 transect surveys were conducted at 20 sites in Kane'ohe Bay, and overall survey areas were approximated to determine the maximal and average abundance of *L. reevii* per m<sup>2</sup> (Figure 14). Surveys were conducted in 2007 to compare with the 2004 findings.

#### **Materials and Methods**

In 2007, 71 sites were surveyed to assess the presence/absence of *L.reevii*; transects were conducted only at sites where *L. reevii* was found to be higher than "Rare" on the DACOR scale (=>20 observed in 20 minute presence/absence surveys). In total, 24 transect surveys were conducted; of these 10 were done at or near sites surveyed in 2004, and presence/absence surveys were compared for the remaining 10 survey sites. In order to compare both survey years, average abundance data from 2004 were converted to the actual numbers observed in each survey area.

#### Results

In 2004, an area of approximately 2,950 m<sup>2</sup> was surveyed with a maximum density of 4 *Lingula*/m<sup>2</sup> and site densities ranging from of 0.01 to 1.4 individuals per m<sup>2</sup> at the 20 sites surveyed (Figure 15; Biol 403 students; Hunter et al., in review). In 2007, within the 71 sites surveyed, *L. reevii* densities ranged from 0.06 to 0.94 individuals/m<sup>2</sup>, with the highest abundance at Site 1B on the dredged reef southeast of Coconut Island (Figures 15 and 16; 206 individuals seen within a 220 m<sup>2</sup> survey area).

The total number of *L. reevii* has dramatically decreased during the last three years (Figure 16). The total number of individual *L. reevii* observed across the 20 sites in 2004 was 2,802 in approximately 2,950 m<sup>2</sup>, while in 2007, only 398 individual *L. reevii* were observed within approximately 390,778 m<sup>2</sup> (Table 1). No *L. reevii* were observed during qualitative

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surveys near Chinaman's Hat in the North Bay in 2004. In 2007, low densities of *L. reevii* were observed in the South Bay and a small number of individuals were observed in the North Bay west of Chinaman's Hat (Figure 17).

In 2004, researchers targeted the southern end of Kane'ohe Bay, as previous studies had observed *L. reevii* densities of up to 500 individuals/m<sup>2</sup> with an average of 25-50 individuals/m<sup>2</sup> in this region (Worcester, 1969). The number of individual *L. reevii* in Kane'ohe Bay in 2007 appears to have decreased at most survey locations, although an increase in abundance was observed at Site A1 (Pyramid Reef) and Site 1B, a dredged reef southeast of Coconut Island (Figures 15, 16). *L. reevii* was observed in high abundance at many of these sites in 2004 (Figure 14, Table 1). For example, highest abundance of *L. reevii* observed in 2004 was at Site B2 (Figure 15) where 852 individuals were observed within 3 x 50 m transects; only one individual was observed within 23, 936 m<sup>2</sup> surveyed in this location in 2007 (Figure 14). In 2007, no *L. reevii* were seen at 10 of the 14 survey sites in South Kane'ohe Bay. Of particular note is the apparent disappearance of *L. reevii* at CI1 (Coconut Island), a site of fairly high abundance in 2004. In 2007, this area was dominated by callianasid shrimp mounds and no *L. reevii* were found.



Figure 14. Map of Kane'ohe Bay with *L. reevii* survey locations in 2004. Fringing reefs are labeled in alphabetical order (A through M) from north to south within Kane'ohe Bay. Patch reef sites follow the convention of Roy (1970). Other abbreviations are as follows: CH = Chinaman's Hat, CI = Coconut Island, and SB = Sandbar.



Figure 15. *Lingula reevii* abundance in Kane'ohe Bay May/June 2004; see Figure 14 for site names.



Figure 16. *Lingula reevii* abundance in Kane'ohe Bay in May/June 2007; see Figure 14 for site names.



Figure 17. The abundance of *L. reevii* at locations in Kane'ohe Bay surveyed in 2004 and 2007.

Table 1. The overall abundance of *L. reevii* and area covered  $(m^2)$  at each quantitative survey location in 2004 and 2007 (area of presence/absence surveys not included).

Survey	Abundance	Abundance	Area m <sup>2</sup>	Area m <sup>2</sup> 2007
Location	2004	2007	2004	
A1	60	155	125	240
A2	0	0	125	27,796
A3	39	0	125	34,776
B1	43	11	150	307,116
B2	852	1	150	23,936
B3	90	0	125	270,956
C1	226	6	150	23,248
C2	279	0	175	N/A
D	306	0	250	49,623
E1	21	0	125	N/A
E2	4	0	200	N/A
E3	80	0	125	22,412
J1	10	1	125	31,073
J2	2	0	125	43,848
L1	54	0	125	18,256
L2	30	0	125	24,830
1A	90	0	75	N/A
1B	127	206	75	220
SB1	44	15	100	240
CI1	441	0	375	32,100
СН	0	3	N/A	375
Total	2798	398	2950	390,778

#### C. The Distribution of Lingula reevii in Kane'ohe Bay as Affected by Water Depth

#### Introduction

Among other habitat characteristics, the influence of water depth on *Lingula reevii* abundance was assessed and analyzed. Previous reports (Worcester, 1969) stated that *L. reevii* was historically found in abundance on shallow reef platforms predominantly in the southern sector of Kane'ohe Bay, in an average depth of 0.5 m (Worcester, 1969) but was not found at depths greater than 10 m. It has been speculated that both sediment type and predation at a depth of 10 m or more are not conducive to *L. reevii* (Worcester, 1969).

In May/June 2007, the presence, abundance and distribution of *Lingula reevii* at 71 sites throughout the bay were assessed to address the relationship between *L. reevii* and water depth.

#### Methods

#### Field measurements

Water depth measurements were taken at 71 survey sites throughout the bay with the use of a water proof depth sounder (Speedtech Instruments 60310). The results of the bottom depth were recorded in feet and inches, and later converted into meters (m) for analysis.

Water depth was recorded at each site; in areas in which there was a larger abundance of *Lingula reevii*, water depths were recorded every 10 m along transects.

#### Results

*Lingula reevii* were present at 24 out of 71 sites surveyed within Kane'ohe Bay. These sites included fringing reef, deeper dredged reefs, sandbar and patch reef habitats throughout the three sections of the bay, (North Bay, Mid Bay and South Bay.) Of these 26 sites, *L. reevii* were present in water depths ranging from 0.5 m to 4.5 m. Because field surveys were conducted via snorkeling, water depths greater than 5 m were not surveyed.

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Figure 19: Average abundance ( $\pm$  S.E.) of *Lingula reevii* within 0.2 m increments of water depth among the four different reef types present in Kane'ohe Bay surveyed between 05/14/2007 - 06/15/2007.

#### Fringing Reefs

Eleven fringing reefs were surveyed totaling an estimated 876,404 m<sup>2</sup> in area, of which eight sites had *Lingula reevii* present. Highest *L. reevii* abundance was between 0.6 - 1.9 m depth. (Figure 19). The standard error associated with maximal abundance at 0.6 m is a attributable to the wide range in number of individuals (1 to 143) within sites surveyed. Depths greater than 1.9 m were not found on the fringing reef flats; reef slopes were dominated by hard coral cover and not conducive to the presence of *L. reevii*.

#### Dredged Reefs

Six deeper dredged reefs were surveyed totaling an estimated 239,757 m<sup>2</sup> in area, of which five sites contained *Lingula reevi*. Because these reefs were dredged to an approximately uniform depth during WWII, water depth was relatively constant at 3.0- 4.5 m. *L. reevii* abundance was relatively high on these reefs, with the highest at 3.8 m depth (Figure 19).

## Sandbar

Six sites along the leeward slope of the sandbar (survey area totaling  $43,337 \text{ m}^2$ ) all showed the presence of *Lingula reevii*. The cumulative distribution of *L. reevii* abundance was highest at approximately 2.4 m depth. Survey depths ranged from 0.6 m to 3.2 m down the sandy slope.

## Patch Reefs

Of the 805,260 m<sup>2</sup> of patch reefs surveyed throughout Kane'ohe Bay, *Lingula reevii* was present on only six patch reefs. *L. reevii* was present in highest abundance between depths of 1.8 - 3.4 m.

#### D. The Distribution of Lingula reevii as Affected by Sediment Grain Size

#### Introduction

The distribution and abundance of *Lingula reevii* in Kane'ohe Bay may be affected by sediment type, grain size, and sediment depth. The sediment depth in which *L. reevii* can burrow, as well as anchor, may depend on these factors (Emig, 1981). Although sediment type was not investigated during this study, sediment grain size samples and sediment depth estimates were obtained at 45 survey sites. At 13 of these sites, multiple samples and depth measurements were taken and the results were averaged for better accuracy.

#### **Materials and Methods**

Sediment grain sizes were compared among samples from locations in Kane'ohe Bay where *Lingula reevii* were present, as well as sites where individuals were absent. During presence and absence surveys, sediment samples were collected by hand in 50 ml plastic tubes. At sites where transect surveys were conducted, multiple samples were collected at evenly spaced intervals along the transects in an attempt to determine optimal sediment grain size for *L. reevii* based on their distribution along the transects.

Each sample was dried at room temperature for at least 48 hours and was subsequently sieved through a series of screens with mesh sizes of 63  $\mu$ m, 125  $\mu$ m, 250  $\mu$ m, 500  $\mu$ m, 2000  $\mu$ m, and 4000  $\mu$ m. The content of each sieve was weighed to determine the percent dry weight of each sediment grain size.

Sediment depth was also obtained at most locations where sediment samples were taken, including sites where *Lingula reevii* surveys were conducted. Sediment depth was measured by inserting a metal wire vertically into the sediment until solid substratum was reached.

#### Results

The predominant grain sizes in locations where *Lingula reevii* was most abundant included a range between 125-500  $\mu$ m (Figure 20). Sediment grain size profiles showed the largest grain sizes occurred at sandbar locations, whereas small grain sizes were more dominant on fringing and patch reefs (Figure 21).

There was no significant relationship between mean sediment depth and *Lingula reevii* abundance (Figure 22, p= 0.376  $r^2$ = 0.0183).



Figure 20. Mean abundance (± S.E.) of *L. reevii* with varying dominant sediment grain size.



Figure 21. Distribution of sediment grain sizes at seven locations in Kane'ohe Bay where transect surveys were conducted to determine the abundance of *L. reevii*.



Figure 22. Relationship between mean sediment depth and *L. reevii* abundance at 43 sites in Kane'ohe Bay.

<u>E. Co-occurrence of *Lingula reevii* with Other Invertebrates, Reef Fish, and Benthic Habitat Types.</u>

#### Introduction

Surveys were conducted to determine if the presence or absence *Lingula reevii* showed correlations to the presence of other benthic organisms or bottom type.

#### **Materials and Methods**

Observations of the relative abundance of other macro-invertebrates (crab and shrimp burrows, featherduster polychaetes, sea cucumbers), reef fishes (scarids and acanthurids), benthic gobies, and habitat type were made at each site using a DACOR (Dominant, Abundant, Common, Occasional, Rare) abundance estimation method. Habitat types were characterized as sand, sand/rubble, sand/coral, sand/rubble/coral, sand/seagrass, algae, or other. Four students collaboratively estimated the relative abundance of organisms and bottom type at each site.

#### Results

There was an inverse relationship between the presence of major benthic invertebrates (shrimp, crabs, featherduster worms, and sea cucmbers) and *Lingula reevii* at the survey sites (Fig. 23). There was a similar effect for reef fish and benthic goby species (Fig. 24). Since *L. reevii* burrow in the sediments, they were most abundant in sandy habitats (Fig. 25). *L. reevii* were rare in areas dominated by other substrata such as coral rubble or seagrass.



Figure 23. Common invertebrates found in Kane'ohe Bay in relation to the number of *Lingula reevii* present in 2007 surveys.



Figure 24. The most frequent fish types in Kane'ohe Bay in relation to the average number of *Lingula reevii* present in 2007 surveys.



Figure 25. Different benthic habitats in relation to the number of times *Lingula reevii* was present in Kane'ohe Bay in 2007 surveys.

## F. <u>Co-occurrence of Alien Invasive Algae and Abundance of *Lingula reevii* in Kane'ohe Bay Introduction</u>

Alien species can affect coral reef ecosystems and alter biodiversity (Pandolfi et al., 2005). Three red algae (*Kappaphycus* spp., *Gracilaria salicornia*, and *Acanthophora spicifera*) have been introduced to the Hawaiian Islands over the past 50 years, the former two for research on their economical potential and the latter was unintentional. Agar and carrageen and are commercial products of *G. salicornia* and *Kappaphycus* spp., respectively, with a value of about US\$270 million per 100,000 tons (McHugh, 2002). By the mid-1990's, *Kappapycus* spp. were spreading at about 250 meters per year, while *G. salicornia* was spreading at 280 meters per year (Rodgers & Cox, 1999) from their initial site of introduction (Coconut Island) in Kane'ohe Bay. These growth rates allowed the algae to spread over 6 km for *Kappaphycus* spp. and about 4 km for *G. salicornia* since their 1974 and 1978 introductions. Rodgers and Cox (1999) also reported that *Kappaphycus* spp. was present throughout the Mid and South Bay while *G. salicornia* was present in the South Bay and portions of the Mid Bay.

*A. spicifera* was introduced after the 1950's at Pearl Harbor. Its ability to grow and reproduce sexually makes it more genetically diverse and more resistant to control efforts than clonal species (O'Doherty & Sherwood, 2007). *A. spicifera* can easily fragment and snag onto benthic substrates or other macroalgae and has a greater potential for dispersal (Smith et al. 2002).

#### Methods

Twenty minute presence/absence surveys for *Lingula reevii* were conducted at 51 sites throughout Kane'ohe Bay. Four groups of paired surveyors quantified flora, fauna, and substratum abundance. The invasive red algae, *Kappaphycus*, *Gracilaria*, and *Acanthophora* were identified by sight, while the more inconspicuous species (e.g. *Hinksia*) were collected for later laboratory identification.

#### Results

Surveys conducted during the present study indicated a more extensive distribution of invasive algae in Kane'ohe Bay than previous reports. *Kappaphycus* spp. was abundant in the Mid and North Bay with patches in the South Bay on patch and fringing reefs (Figure 28). *Kappaphycus* spp. was not seen at Coconut Island during these surveys. *Gracilaria salicornia* was abundant in the South Bay with patches in the Mid and North Bay (Figure 29). *Acanthophora spicifera* had patchy distributions with dense patches in some areas of the Bay (Figure 30).

*Lingula reevii* were in greater abundance where invasive algae were low or absent (Figure 27). *L. reevii* were found in the sediment, usually on reef flats, sand patches or at the sandbar. Algae abundances were estimated for the patch reef, fringing reef, or sandbar regardless of the distribution or abundance of *L. reevii*. At Patch Reef 15 in the Mid Bay, 52 *L. reevii* were found in a 190 m<sup>2</sup> sandy patch on the reef slope. *Gracilaria salicornia* and *Acanthophora spicifera* were occasional on this reef, but were not present in the sandy patch where *L. reevii* was found.



Figure 26. Average *Lingula reevii* per m<sup>2</sup> at all survey sites graphed against abundance of the invasive algae *Acanthophora*, *Gracilaria*, and *Kappaphycus* spp. Highest *L. reevii* abundances correspond with the absence of invasive algae.



Figure 27. Relative abundance of major invasive alien algae in Kane'ohe Bay, May-June, 2007.



Figure 28. Relative abundance of *Kappaphycus/Eucheuma* spp. in Kane'ohe Bay, May-June, 2007.



Figure 29. Relative abundance of Gracilaria salicornia in Kane'ohe Bay, May-June, 2007.



Figure 30. Relative abundance of *Acanthophora spicifera* in Kane'ohe Bay, May-June, 2007.

#### G. Effects of Recreation Activities at the Sandbar, Kane'ohe Bay, on Lingula reevii

The Sandbar in Kane'ohe Bay has long been used by local residents, visitors, and commercial recreational operators. While local residents use the Sandbar mainly on weekends, commercial boats come out daily (except for Sundays and holidays), generally with 50+ people per trip. The commercial operators set up volleyball nets and encourage walking and snorkeling on the Sandbar.

A senate bill proposed in 2004 instructed the Hawaii Division of Land and Natural Resources (DLNR) to set up regulations and restrictions on commercial recreational use of Kane'ohe Bay (Figure 31), including the Sandbar (KHNL News, 2004). These restrictions remain a topic of controversy for both Native Hawaiians and local residents for many reasons (Honolulu Advertiser, 2006). An additional concern is the ecological impacts of activities on the Sandbar, particularly to NOAA's Species of Concern, *Lingula reevii*. If the Sandbar is shown to be an important habitat for *L. reevii*, it may be necessary to better enforce or require additional regulations prohibiting recreational activities to take place there in addition to the current commercial management zones.

#### Methods

ArcGIS 9.2 was used to map the results of *Lingula reevii* surveys in comparison to the State's Ocean Recreation Management Zones in Kane'ohe Bay (Figure 32).

#### Results

The highest total numbers of *Lingula reevii* in Kane'ohe Bay Ocean Recreation Management Zones were found in Zone H (Figure 33), an area prohibited for ocean recreation of most types and including the Sandbar (an important habitat for *L. reevii*). However, Zone G had a higher abundance (per m<sup>2</sup>) of *L. reevii* (Figure 34) as the result of a dense patch of *L. reevii* in a small area (~ 190 m<sup>2</sup>) at Patch Reef 15 (Figure 35). *L. reevii* found at Patch Reef 15 were at depths of approximately 3-4 m, compared to the Sandbar where *L. reevii* was found at approximately 1-3 m deep. These greater depths may protect *Lingula* from trampling due to recreational activities at the Sand Bar. It would be instructive to find out how strictly zoning regulations are followed in Kane'ohe Bay, because on more than one occasion jet skis and other thrill crafts were observed in the prohibited zones during our surveys. Recreational and commercial boats were often seen anchored on the Sandbar in Zone H, an area in which commercial use is prohibited.

During one of the surveys of the Sandbar, we entered an area occupied by a commercial boat and jet skiers. We were asked by the commercial operator to avoid the area. Preliminary presence/absence surveys in this area (Figure 36) had shown a relatively high estimated abundance of *Lingula reevii*, but subsequent relocation of quantitative transects to avoid the jet skiers (but still within the commercial use area) yielded a total of only 16 *L. reevii* in 240 m<sup>2</sup>.

Another interaction with recreational activities in Kane'ohe Bay occurred during a survey conducted near a large commercial boat on the Sandbar. Due to the fact that we arrived prior to the commercial vessel, we were not asked to relocate; however, during the presence/absence survey, a volleyball net was set up and approximately 40 people were observed standing and jumping on the shallow site. After the commercial boat left, we surveyed the volleyball area and found no *Lingula reevii*, whereas 50 m away up to 30 *L. reevii* were found in 5-10 minute presence/absence surveys.





Zone K Recreational Thrill Craft





Figure 32. Number of surveys conducted in each Kane'ohe Bay Ocean Recreation Management Zone (as shown in Figure 31).



Figure 33. Number of *Lingula reevii* found in each Kane'ohe Bay Ocean Recreation Management Zone (as shown in Figure 31).



Figure 34. Area (m<sup>2</sup>) in each Ocean Recreation Management Zone (as shown in Figure 31).



Figure 35. Abundance (per  $m^2$ ) of *L. reevii* within each Ocean Recreation Management Zone (as shown in Figure 31).



Figure 36. Presence or absence of *L. reevii* within each Ocean Recreation Management Zone. Background colors delineate zones as shown in Figure 31. Red dots indicate absence, green dots indicate presence.

#### Discussion

*Lingula reevii* has a disjunct distribution and is only found in Kane'ohe Bay (Emig, 1978), Ambon, Indonesia (Cals and Emig, 1979) and Japan (Emig, 1997). There are no data on the relative abundance of this species in Indonesia or Japan, nor has it yet been confirmed by genetic analyses that these three populations are all representatives of the same species. In addition, very little is known about the physiology or reproduction of *L. reevii*. A number of factors may have contributed to its continued decline in Kane'ohe Bay, such as the proliferation of mat-forming invasive algae (i.e. *Kappaphycus spp., Eucheuma spp., and Gracilaria salicornis)* which are now dominant components of the benthic habitat in many of the areas surveyed. Recreational activities may also be having an impact on the abundance of *L. reevii* in sandbar habitats.

*Lingula reevii* was found in lower overall abundance per m<sup>2</sup> than previously reported  $(0.9/m^2 \text{ in } 2007; 500/m^2 \text{ in } 1969)$ . *L. reevii* abundance at 26 sites surveyed throughout the Bay was generally at deeper depths than those previously reported. Worcester (1969) found that *L. reevii* were most abundant at an average depth of 0.5 m; results from the 2007 surveys showed a higher abundance on reef flats at depths of 0.6-1.9 m, at 2.4 m at the Sandbar, at 3.8 m on dredged reefs, and 1.8-3.4 m on patch reefs. The relationship between depth and average abundance of *L. reevii* in Kane'ohe Bay appears to have shifted.

Such a downward movement of *Lingula reevii* abundance within Kane'ohe Bay may be attributed to stressors such as invasive algal species and/or anthropogenic effects. Particularly at the Sandbar sites, the high impact of human and motorized vessel traffic in the shallower areas may account for the downward shift in *L. reevii* abundance.

Sediment grain sizes varied within Kane'ohe Bay from <63- 4000  $\mu$ m, with the most dominant sizes between 125-500  $\mu$ m. The dominant grain sizes varied among the sandbar, fringing reefs, patch reefs, and dredged reef locations. Sandbar locations averaged a large dominant grain size of 500  $\mu$ m, and also supported the largest abundances of *Lingula reevii*. Patch reefs had a smaller dominant grain size of 125  $\mu$ m and lower abundances of *L. reevii*. Average sediment depth showed no significant effect on the abundance of individuals at sites surveyed within Kane'ohe Bay.

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The inverse relationship between the abundance of *Lingula reevii* and other benthic species may be due to competition for space within the sand or bioturbation. Seagrasses may also outcompete *L. reevii* for space in some areas.

Invasive algae may also compete with *Lingula reevii* for available substrate. *Kappaphycus, Gracilaria*, and *Acanthophora* are capable of forming dense mats on benthic substrates that can alter the water flow for the filter-feeding *L. reevii*. With spreading rates of 250 m/year and 280 m/year for *Gracilaria salicornia* and *Kappaphycus* spp. respectively, the invasive algae have the potential to rapidly alter the coral reef ecosystem in Kane'ohe Bay. With high growth rates, these algae are able to take up nutrients and convert it into biomass. This may reduce the amount of nutrients in the water column available for other organisms including microalgae upon which (in part) *L. reevii* feeds.

For the past 200 years, Kane'ohe Bay (particularly in the South Bay) has undergone dramatic changes in surrounding watershed development and land use; reefs in the Bay have been dredged to accommodate ship traffic, seaplane landing sites, and housing projects (Hunter and Evans, 1995). With an increase in population size, secondary treated sewage was discharged into the South and Mid Bay from the mid-1940's until the late 1970's. Runoff from development and sewage outfalls delivered high-nutrient waters directly into the bay, impacting water column and benthic habitats where *Lingula reevii* once thrived (Gulko, 1998).

There is certainly some ecological irony in that historic nutrient subsidy from sewage discharge may have helped to support the previously high population densities of this rare species in Hawaii. Management implications are that protective measures be implemented to prevent further declines in *L. reevii* abundance. These may include removal of alien algae from *L. reevii* habitats and the initiation of *ex situ* cultures, as is currently being attempted at the Waikiki Aquarium with seven specimens collected in June, 2007, from Site 1B.

# II. Current Abundance and Distribution of *Montipora dilatata* in Kane'ohe Bay, Oahu,

#### Introduction

The scleractinian coral genus *Montipora* is found throughout the tropical regions of the world. These corals are commonly known as 'rice corals' due to their characteristic tubercules or verrucae (Veron, 2000). Colonies can form submassive, laminar, encrusting, branching forms, or any combination depending upon the species and its environment (Veron, 2000).

*Montipora dilatata* (Studer 1901) is endemic to the Hawaiian Islands. It has only been recorded from the Northwestern Hawaiian Islands and Kane'ohe Bay, Oahu, and it is still uncertain whether these are the same species (Fenner, 2005). Colonies of *M. dilatata* can range from foliose to massive or glomerate, but lack the prominent projections often found in other species of *Montipora*. Calices are small at 0.5 - 1 mm in diameter (Maragos 1977; Veron 2000), well defined and have characteristic thin, laminar rings or collars surrounding each calyx (Maragos, 1977).

*M. dilatata* may be commonly confused with *M. turgescens*, which has a coenosteum also lacking elaborations and has similar habitat and growth forms (Veron, 2000); it is also similar to congeners *M. incrassata* and *M. flabellata* (Veron, 2000).

#### **Problem Statement**

The National Marine Fisheries Service (NMFS) defines a Species of Concern (SOC) as a species that is not being actively considered for listing under the Endangered Species Act (ESA) but for which significant concerns or uncertainties regarding its biological status and/or threats exist (69 FR 19975). The purpose of the NMFS SOC Program is to conduct proactive conservation activities under the ESA to preclude the listing of future species. One of the Pacific Islands Region Species of Concern is the Hawaiian reef coral, *Montipora dilatata*.

*M. dilatata* has been recorded in: 1) the main Hawaiian archipelago in Kane'ohe Bay, Oahu; and 2) in the Northwestern Hawaiian Islands. In 2000, surveys of *M. dilatata* conducted by J. Maragos, D. Fenner, D. Gulko, and C. Hunter identified only three colonies in Kane'ohe Bay, where it formerly was reputed to be more abundant (Maragos 1977). Habitat degradation as a result of sedimentation, pollution, alien/invasive algae species, and

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its historically limited distribution may be contributing factors to the apparent decline of this species in Kane'ohe Bay.

#### **Specific Tasks**

The purpose of this study was to perform the following tasks as stipulated by a contractual agreement with NMFS: conduct extensive surveys of all suitable habitats in Kane'ohe Bay; systematically map *Montipora dilatata* and quantify the current population size; quantify the occurrence of alien/invasive algae; and characterize and photo-document the habitat types (e.g., substratum, depth, and rugosity) to improve understanding of the species' habitat requirements.

#### Methods

Surveys for the presence of *Montipora dilatat*a were conducted throughout Kane'ohe Bay from 16 May-13 June, 2007. The occurrence of alien/invasive algae was also recorded.

Fragments of *M*. cf. *dilatata* were collected under the auspices of David Gulko (Hawaii DLNR-DAR), as well as several fragments of *M*. *capitata* for comparison.

A subsample of each fragment was preserved in 95% ethanol for genetic analysis; the remainder was placed in a 50% bleach solution overnight. Samples were then rinsed in fresh water and dried before measurements and photographs were taken. Comparisons were made of calical structure, size, and coenosteum structure.

#### Results

Presence/ absence surveys were confounded by an apparent spectrum of *Montipora* species/ morphotypes within Kane'ohe Bay. *Montipora* species showed high morphological plasticity, making positive field identifications difficult.

• Microstructure analysis



Figure 37: Dissection microscope views of calices of *Montipora capitata* (average polyp diameter: 1mm, n=12) (A), *Montipora* species unknown (average polyp diameter: 0.5mm, n=12) (B), and *Montipora* cf. *dilatata* (average polyp diameter 1mm, n=12)(C). Species identifications based on gross morphology before microscopy.

In the photographic and polyp size comparisons, there were only two samples with polyps averaging near 0.5 mm (Figure 37: B). Several types of calyx structures were identified among the 23 samples collected. The 0.5 mm calyx diameter samples appeared to have a 'star'-like calyx structure, with a finely ridged coenosteum (Figure 37: B).

The remaining fragments with calices typically of approximately 1 mm could be divided into two groups:

Group 1: Verrucae or tubercules present, ovoid 'flower'- shaped calices with a more robust coenosteum (Figure 37 A).

Group 2: Verrucae and tubercules absent, 'snowflake'- like calyx structure with a finely structured coenosteum (Figure 37 C).

#### Discussion

Surveys of *Montipora dilatata* in 2000 by J. Maragos, D. Gulko, D. Fenner, and C. Hunter located only three colonies of *Montipora dilatata* in Kane'ohe Bay, Oahu, Hawaii. The current abundance of *M. dilatata* was unable to be determined due to our inability to reliably identify this species in the field.

The differences of opinion (among these and other coral experts) as to definitive characteristics of *M. dilatata* led to collection of samples for further genetic and microstructure analysis.

Microstructure analysis found only two samples which showed the characteristic 0.5 mm polyps as characterized by Veron (2000). There appeared to be a difference between the samples identified as *M. capitata* (smaller polyps), and between the *M.* cf. *dilatata* based on polyp size, shape, and coenosteum structure.

To gain a better understanding of this group of species, scanning electron micrographs of each sample should be obtained, in addition to genetic analysis. Genetic differences can then be matched to morphological differences, if possible, and hopefully used as an aid to field identifications.

At the sites where colonies were located that were judged most likely to be *M. dilatata*, the benthic habitat was relatively low in alien/ invasive algal cover, and was characterized by high water motion and high rugosity (personal observations).

One theory for the range of morphological diversity in *Montipora* is that two or more species may be producing hybrids (J. Maragos, personal communication). The species differences remain to be investigated, but include *M. dilatata*, *M. capitata*, *M. incrassata*, *M. turgessens*, and perhaps others. There are currently up to 12 *Montipora* species known in Hawaii, creating many possibilities for hybrid combinations (Veron, 2000).

An important factor in this questions is to determine if there is an overlap in species spawning (Heyward and Stoddart, 1985; Hunter, 1988), both spatially (relative locations of colonies and level of gametes in the water column) and temporally (lunar phase, time of day or night). Gamete compatibility and the viability of larvae produced will also play a role in the success of any hybrids formed.

There is also the problem of species misidentification. It is still unknown whether *M*. *dilatata* and *M*. *turgescens* are the same species, or whether the *M*. *dilatata* reported from the

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Northwestern Hawaiian Islands is in fact the same species as reported in Kane'ohe Bay (Fenner, 2005).

To solve this problem, and to aid any future work with *Montipora*, an in-depth study of the *Montipora* species present, their genetics, morphology, and ecology needs to be carried out. It is through a combination of techniques that reliable characteristics will be identified to aid field identification of *M. dilatata*, and consequently to investigate its abundance and distribution.

In the meantime, *Montipora dilatata* needs the highest level of protection possible afforded to it. Its abundance may have been previously over estimated and until *M. dilatata* can be reliably identified, its true abundance will remain unknown. It is better to be over-cautious at this time than to risk losing a species unique to Hawaii altogether.

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