Environmental Investigation into impacts of Land-Based Sources of Pollution on Coral Health in West Maui, Hawaii

CRCP Project 502 Interim Report

West Maui, HI has been plagued with reports of poor water quality in the near shore coastal zone, fecal indicators exceeding EPA standards, and algal blooms for over 20 years with a corresponding steady decline in coral cover from 70% (1990s) to 27% (2006). This interim report provides baseline fecal indicator data in dry (22 sites) and rainy (14 sites) seasons and porewater toxicity data for 16 locations on Maui to help clarify the role of wastewater injection wells may play in coral decline and assist in BMP monitoring efforts. This information can help strategically focus costly management efforts on the greatest risk factors.

Interim Report

Environmental Investigation into impacts of LBSP on Coral Health in West Maui, Hawaii

Cheryl M. Woodley[1], Craig A. Downs[2], Lisa A. May[3], Erin Looney[4], Darla White[5] and Kathy Chaston[6]

[1]NOAA National Ocean Service, Center for Coastal Environmental Health & Biomolecular Research, Charleston, SC
[2]Haereticus Environmental Laboratory, Clifford, VA
[3]JHT, Inc. Contractor to NOAA National Ocean Service, Center for Coastal Environmental Health & Biomolecular Research, Charleston, SC
[4]NOAA National Marine Fisheries Service, Pacific Islands Fisheries Science Center, Coral Reef Ecosystem Division, Honolulu, HI
[5]Special Projects Coordinator, Hawaii Department of Land and Natural Resources, Division of Aquatic Resources, Maui, HI
[6]Hawaii Coral Management Liaison and Pacific Watershed Specialist, NOAA Coral Reef Conservation Program, Pacific Services Center, Honolulu, Hawaii

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Executive Summary

Preliminary data from 2011, showed that samples associated with freshwater seeps from Ka'anapali contained detectable levels of oxybenzone and phthalates. Both of these compounds have been associated with sewage and showed up to 40% mortality in coral cell toxicity tests. The purpose of this study was to determine if localized anthropogenic pollutants, including sewage, are impacting specific bays along Maui's West coast. This interim report contains results from surveys of priority sites along Maui's coast for indications of anthropogenic impacts. Bacterial fecal-indicators and potential human and coral pathogens were cultured and enumerated from water column samples, as measure of sewage input into an area. Porewater toxicity tests were conducted using the sea urchin embryo development toxicity tests as an indicator of anthropogenic pollutants. Together these data are being used to identify sites that pose the highest risk to coral health.

Water quality was assessed by quantifying levels of *Enterococcus*, *E. coli* and fecal coliforms. EPA and local health departments use these bacteria as sewage indicators for regulating water quality and beach closures. Total *Staphylococcus and Staphylococcus aureus,* known human pathogens that can be found in marine waters, were also enumerated; in addition, these bacteria are of interest because of concern over some strains having multiple antibiotic resistance (MRSA). Levels of *Serratia marcescens,* a bacteria also associated with sewage and a potential human pathogen, as well as being associated with a Caribbean coral disease, acroporid serratiosis (aka white pox disease) were also tested.

Hawaii's bacterial water quality criteria uses *Enterococcus* as a primary indicator and limits are set at a geometric mean of 35 colony forming units (CFU)/100mL and a single sample maximum limit of 104 CFU/100mL. *Clostridium perfringens* is used as a secondary indicator; however, it was not included due to lack of proper containment facilities in this study. Fecal indicator bacteria and other potential pathogens were surveyed at 22 sites in September 2012 and 14 sites in February 2013 along the West Maui Coast to compare levels in a dry versus rainy season. In September (dry season), none of the sites sampled exceeded the EPA bacterial water quality criteria. In February, however, four sites exceeded the single sample maximum limits and five sites were greater than 35 CFU/100mL. High levels of total *Staphylococcus and Staphylococcus aureus* at most sites were seen in both sampling periods. In February, additional testing for *Staphylococcus* and *Staphylococcus aureus* indicated coral mucus levels were much higher than levels in the adjacent water column. *Staphylococcus* has not been associated with coral disease, however these and other observations warrant closer investigation.

Porewater toxicity tests indicated that 16 of 17 samples tested positive for toxic-associated pollution. Toxicity Identification Evaluations indicated that porewater pollutants were predominantly associated with organic derived pollutants, though samples from three sites retained toxicity after removing the organic-based toxicants, suggesting they contained other toxic components, potentially metals. The results demonstrate that anthropogenic pollutants may be significant factors contributing to coral reef degradation.

Introduction

West Maui, HI has been plagued with reports of poor water quality in the near shore coastal zone (HIDOH 2012), fecal indicators exceeding EPA standards, and algal blooms for over 20 years. A corresponding steady decline in coral cover from 70% (1990s) to 27% (2006) has also been reported. Much attention has been focused on sewage treatment plants and the practice of injection wells in this region. A 2009 USGS study (Hunt & Rosa 2009) provided evidence of a plume, with nearshore freshwater springs containing a plethora of pharmaceuticals and personal care products. Effluent escaping into coastal waters was further supported by an extensive nitrogen isotope (sewage marker) survey throughout Maui, supporting injected effluent from sewage plants as a possible source of the elevated nitrogen levels in the Kahekili area. Opposing arguments cite sediment runoff, natural terrestrial nitrogen sources, fertilizer and legacy agro-chemicals as being responsible for algal blooms and coral decline. Though there is compelling evidence for injection wells causing environmental hazards for public recreational activities and coral health, it is insufficient to clearly link the practice of injection well disposal of effluent as having detrimental effects on coral health.

The purpose of this study is to determine if localized anthropogenic pollutants are impacting specific bays along Maui's West coast. The information obtain from this project will help establish baseline data for comparison with BMP monitoring, as well as to assist in determining the efficacy of sewage treatment system upgrades when they are implemented in West Maui. This information is being shared with NOAA and USACE to inform their watershed management planning and provide Federal (EPA) and state (HDLNR) decision-makers more definitive answers before undertaking costly wastewater improvements to appropriately regulate the wastewater discharge, and manage other pollution sources at the site. The project objectives for Year 1 were to: 1) assess the potential toxicity of sediment porewaters in targeted bays; 2) conduct a Toxicity Identification Evaluation (TIE) on samples showing toxicity; 3) survey West Maui bays for levels and identity of fecal-associated bacteria and other pathogens in dry and rainy seasons; and 4) provide practical training and technology transfer to local resource managers and students in the methods used in this study.

Toxicity Testing

Assessing Toxicity of Sediments in Targeted Maui Bays

Sediment and porewater were collected from 15 sites along the Maui coast (Fig. 1) as well as from additional freshwater seep sites in Kahekili. All samples were collected within 30 meter of shore in areas where coral could be found, even if coral colonies were only marginally found. Samples # 6 and #10 were collected from sites with no observable living coral, although coral rubble was in abundance; indicating it was once coral habitat. Surface sediment samples (containing porewater) were collected using a modified syringe. Syringes without rubber plungers or silicone (e.g., Fisher Scientific #03-377-24) were modified by removing the tip-end of 30ml syringes, leaving an open syringe barrel to serve as a coring device. The collector positioned the syringe on to the surface of the sediment and pulled back on the plunger while pushing the syringe into the sediment to an approximate depth of 3-5 cm. When the syringe was filled, the collector (wearing nitrile gloves) withdrew the syringe barrel, while placing a thumb or two fingers over the opening until the sample could be placed into the Teflon bag (Welch Fluorocarbon, Dover NH), either at the surface or underwater (depending on depth). This process was repeated until sufficient surface sediment was collected for analysis (15-20ml for porewater test; 100-500 ml for TIE). Note that collection can only proceed if the surface sediment remains undisturbed in an area. Sediment samples were stored in PFA-Teflon bags, and frozen at -20°C until used. Samples were frozen with dry ice and shipped on dry ice to the Haereticus Environmental Laboratory in Virginia for analysis. Onsite sea urchin toxicity assays were not feasible in the timeframe available on site.

Site Number	Sampling Location
1	La Perouse
2	Makena Road
3	Makena Park
4	Polo Beach
5	Ulua Beach Park
6	Lipoa Place, Kihei
7	Kalaepohaku Pier
8	Olawalu E.
9	Olawalu W.
10	Lahaina
11	Kahana Bay
12	Honokeana Bay
13	Napili Bay
14	Kapalua Bay N.
15	Kapalua Bay S.

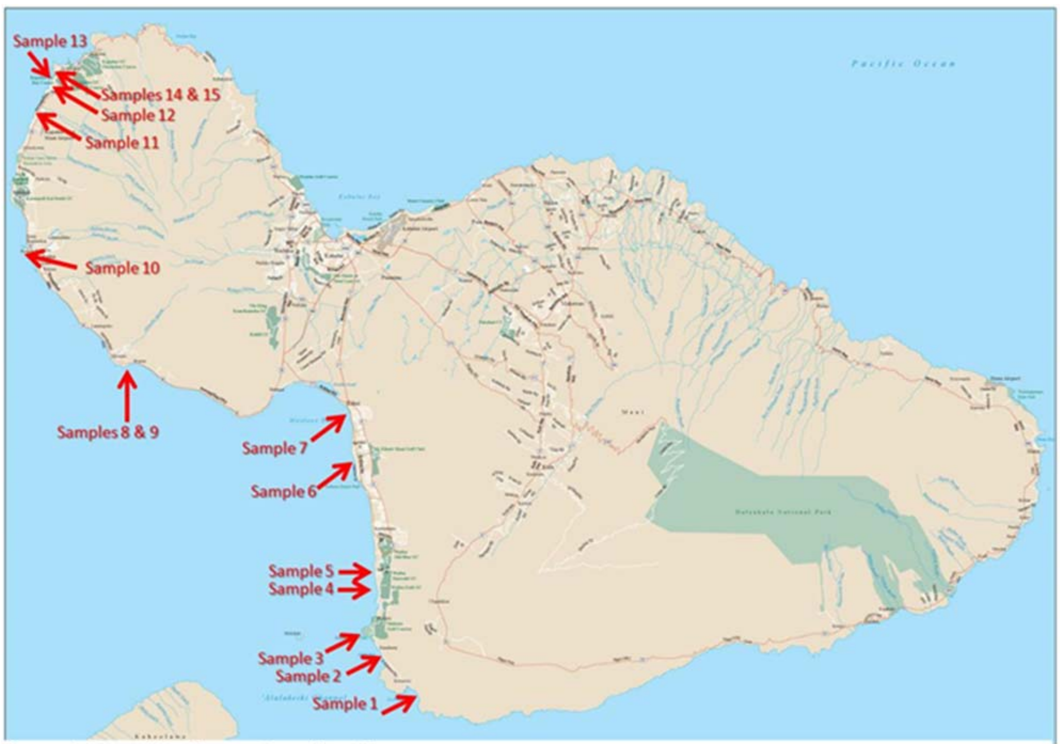
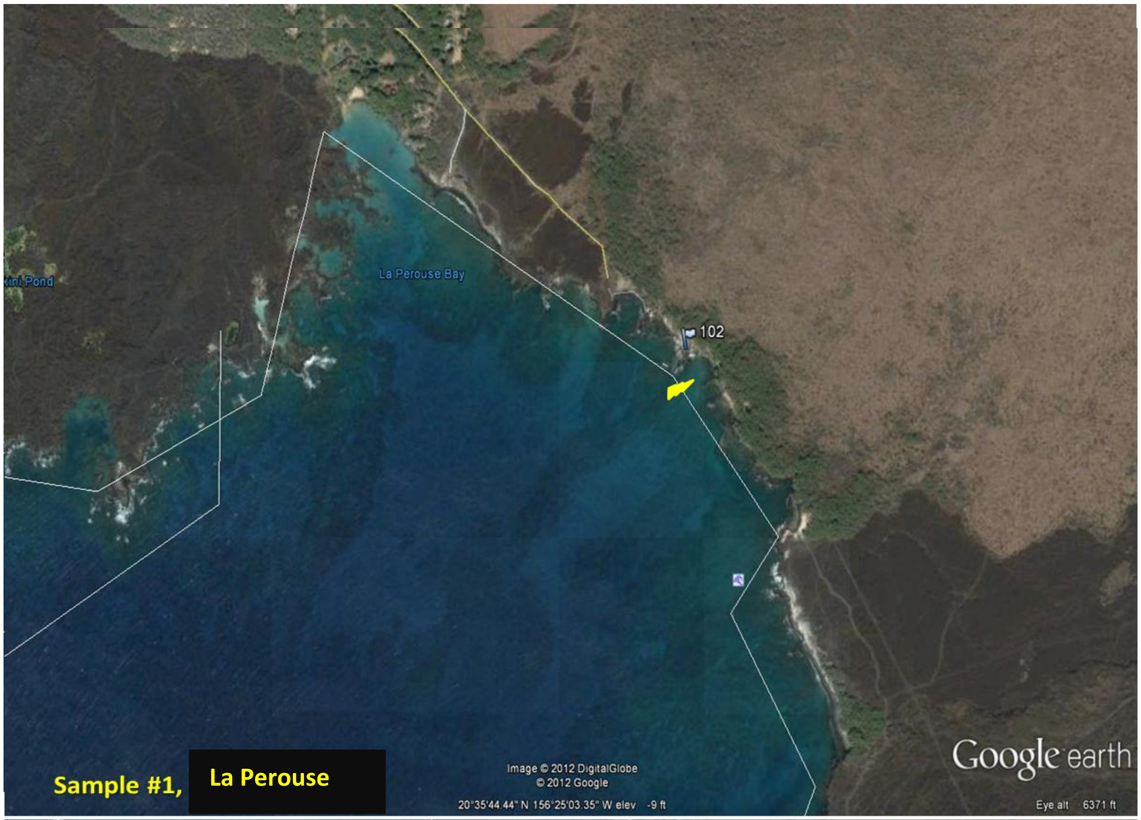


Figure 1 Map of Sediment Sampling Sites.

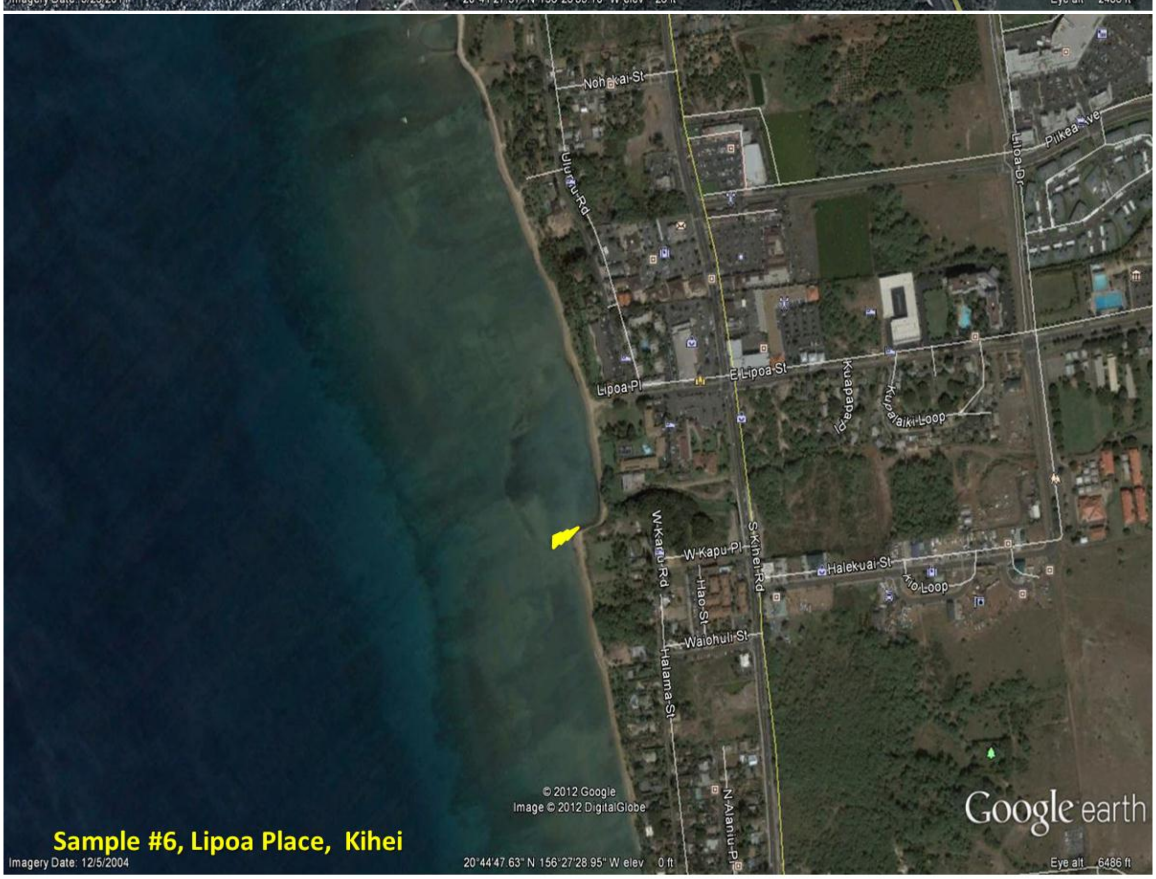
The following 16 Google Earth figures provide aerial images of each sampling site and indicate the specific location within the site that samples were taken.



Sample #3, Makena Park
Imagery Date: 12/20/2006
Image © 2012 DigitalGlobe
© 2012 Google
20°37'59.94" N 156°26'52.14" W elev 0 ft
Makena Rd
Google earth
Eye alt 7503 ft

Sample #4, Polo Beach
Imagery Date: 12/5/2004
Polo Beach Club
A Plus Resorts
Fairmont Kea Lani
Wailea Alanui Dr
Kaukahi St
Manalo St
Waiola Loop
Wailina Pl
Image © 2012 DigitalGlobe
© 2012 Google
20°40'34.48" N 156°26'29.85" W elev 71 ft
Google earth
Eye alt 4310 ft


Sample #5, Ulua Beach Park
Ulua Beach Rd
Hale Alii Pl
Hale Hookipa Way
Wailea Alanui Dr
Wailea Beach Path
Image © 2012 GeoEye
© 2012 Google
Google earth
Imagery Date: 3/23/2011
20°41'27.57" N 156°26'33.10" W elev 26 ft
Eye alt 2486 ft


Sample #6, Lipoa Place, Kihei
Nohokai St
Uluniu Rd
Piikea Ave
Lipoa Dr
Lipoa Pl
E Lipoa St
Kuapapa Pl
Kupalaiki Loop
W Kapu Pl
Halekuai St
Ao Loop
W Kaonu Rd
Hao St
Waiohuli St
S Kihei Rd
Halama St
N Alanu Pl
© 2012 Google
Image © 2012 DigitalGlobe
Google earth
Imagery Date: 12/5/2004
20°44'47.63" N 156°27'28.95" W elev 0 ft
Eye alt 6486 ft

Sample #7, pier at Kalaepohaku

Sample #8 & #9, Olawalu


Sample #10, Lahaina
Hiki Pl
Kapoma St
Nahale St
Hopili St
Keao St
Kapunakea St
Ulupono St
Keawe St
Kupuohi St
Ala Moana St
Front St
Puunoa Pl
Haipaaka Pl
Hinau St
30
© 2012 Google
Image © 2012 DigitalGlobe
© 2012 Europa Technologies
Google earth
Imagery Date: 1/25/2007
Eye alt 5313 ft


Sample #11, Kahana Bay
Kili Nahe St
Akeloa Pl
Kapulu Pl
Hanawai St
Aloalo Pl
Hui Rd E
Hui Rd C
Napilihau St
Hui Rd B
Lower Honoapiilani Rd
30
© 2012 Google
Image © 2012 DigitalGlobe
© 2012 Europa Technologies
Google earth
Imagery Date: 1/25/2007
20°59'03.24" N 156°40'13.18" W elev 71 ft
Eye alt 3894 ft


Honokeana Bay
Lower Honoapiilani Rd
Piilani Ln
Papaua Pl
Hui Rd F
© 2012 Google
Image © 2012 GeoEye
© 2012 Europa Technologies
Google earth
Sample #12, Honokeana Bay


Napili Bay
Lower Honoapiilani Rd
© 2012 Google
Image © 2012 GeoEye
© 2012 Europa Technologies
Google earth
Sample #13, Napili Bay


Namalu Bay
Bay Dr
Ironwood Ln
Ridge Rd
Coconut Grove Ln
Lower Honoapiilani Rd
Kapalua Dr
Napili Bay
Pineapple Rd
Cook Pine Rd
Hui Rd H
Image © 2012 DigitalGlobe
© 2012 Google
Google earth
Sample #14 & 15, Kapalua Bay
20°59'57.14" N 156°39'49.29" W elev 91 ft
Eye alt 4867 ft


30
Kai Ala Dr
Honoapiilani Hwy
Image © 2012 GeoEye
© 2012 Google
Google earth
Sample #16, Near North Seep
20°56'19.32" N 156°41'27.19" W elev 9 ft
Eye alt 2478 ft

Porewater Toxicty Assays Using Sea Urchin Embryos

Sea urchins (*Lytechinus variegatus*) were purchased from Gulf Specimen Marine Laboratory (Panacea, FL). Sea urchins were spawned and viable egg and sperm were mixed to produce embryos. The sea urchin embryo toxicity assay was conducted in a PTFE-Teflon® 24-well microplate.

Porewater from the surface sediment was collected by gravity extraction. Porewater was tested for salinity, pH, calcium concentration, and where porewater needed it, samples were corrected to ensure that these three factors did not confound the results.

Two milliliters of porewater from each sample were placed into a well; and four wells filled with porewater from each site. Between 20-40 embryos were placed in each well. Embryo/seawater volume did not exceed 200 microliters to each well. Sea urchins embryos were incubated at 26°C for 56 hours until control-artificial seawater treatment reach early pluteus stage. Sea urchin plutei were then scored based on deformities, developmental arrest, or mortality. No toxic effects were observed with the artificial seawater controls for sea urchin embryos (i.e., mortality, arrested development or deformaties).

Kahekili samples, south of the south seep and WNA 1-3 were provided to Haereticus for analysis by the Hawaii Ridge-2-Reef coalition, EPA (Wendy Wiltse) and NOAA (Kathy Chaston/Cheryl Woodley).

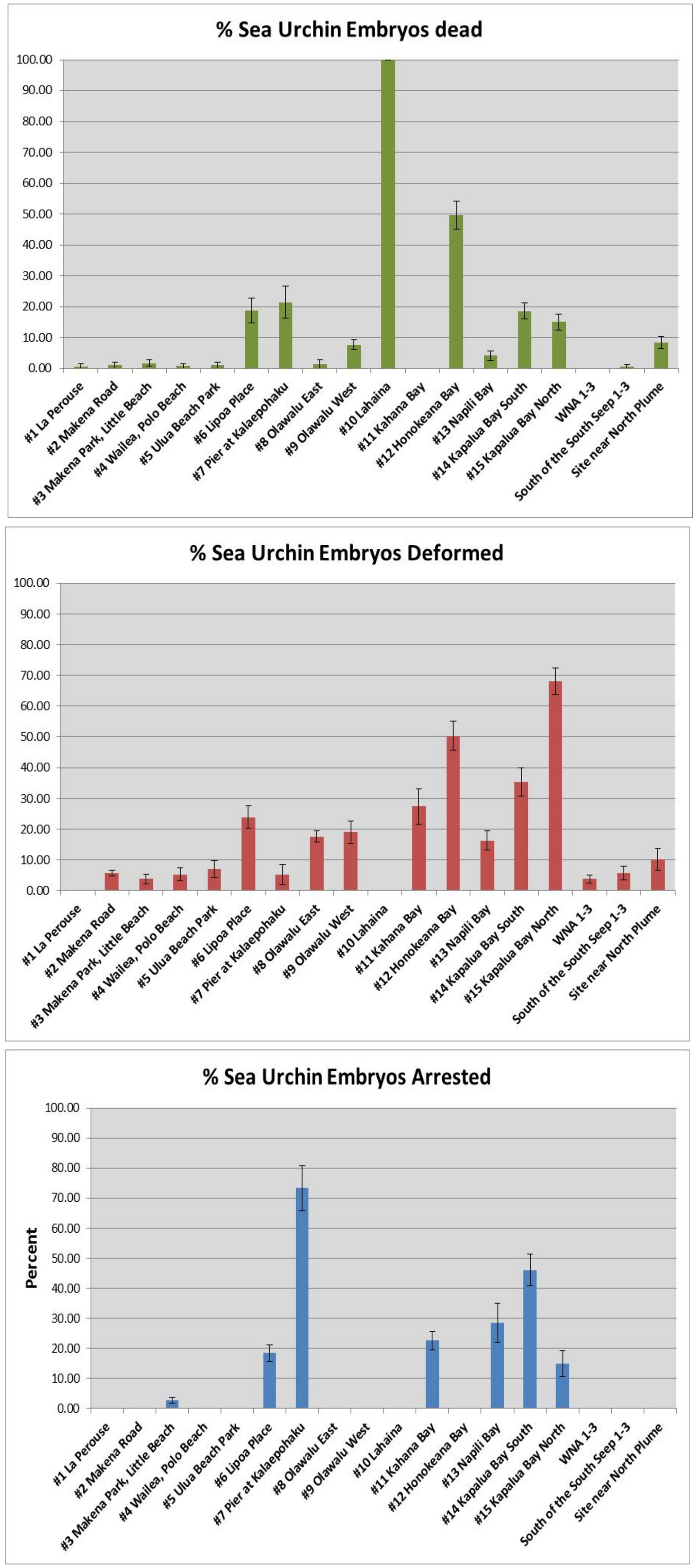


Figure 1 Porewater toxicity tests for the 16 sites of West Maui showing site toxicity (percent mortality, deformities and arrested development).

All sites but La Perouse #1 tested positive for toxic-associated pollution (Fig. 2). Samples displaying the highest mortality were from Lahaina #10 (100%) and Honokeana Bay #12 (49.65%). Honokeana Bay also displayed marked toxicity with deformed plutei (50.35%) along with samples from the north side of Kapalua Bay #15 (68.04%). Samples from the south side of Kapalua Bay #14 showed a different pattern with 46.07% arrested in development, 35.3% deformed and similar levels of mortality with samples from the north side of the bay. Samples from the pier at Kalaepohaku, however, showed the highest level of developmental arrest (73.38%) among all sites tested. The three samples tested from Kahekili showed less than 10% of the embryos affected by exposure to the porewaters, indicating relatively little toxicity in those samples compared to that of samples from other locations. Among the Kahekili samples tested, the highest toxicity was associated with samples from a site near the North Plume with 10.2% deformed embryos and 8.5% mortality.

Based on the porewater findings and the availability of porewater, a Phase I Toxicity Identification Evaluation (TIE) was conducted. This included sample #6-Lipoa Place, #7- Pier at Kalaepohaku, #8- Olawalu East, #9-Olawalu West, #12- Honokeana Bay, #15- Kapalua Bay North and WNA 1-3 which is located near the North Plume at Kahekili.

Toxicity Identification Evaluation – Sea Urchin Embryos

Porewater was treated with various solid phase extraction columns to determine the general category of the pollutant that is causing toxicity. Toxicity reduction treatments had to be prioritized to work within the available porewater for each sample; therefore, not all toxicity reduction treatments could be conducted. A C18 treatment was used for all samples because of its ability to bind a wide variety of organic compounds. These columns bind nonpolar to moderately polar compounds (e.g., organics). For three sites, the AX column was used. This column has “nonpolar and cationic characteristics for improved analysis of acidic drugs and metabolite”, it is a mixed mode with C8 and quaternary amine, anion exchange, nonpolar

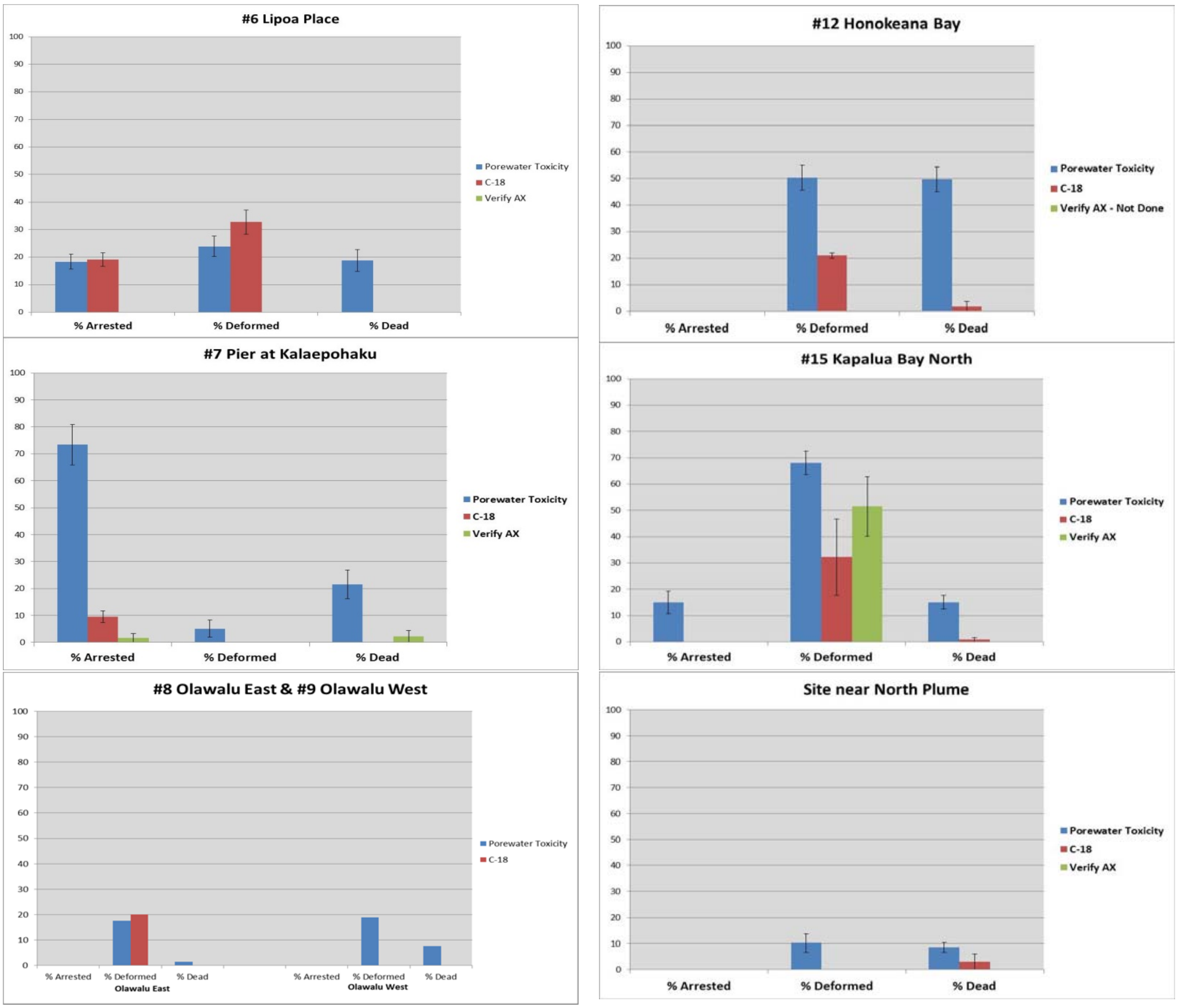


Figure 2 Phase I TIE. Porewater from 7 sites was filtered with either a C18 or Verify AX column to remove potential toxicants and eluents were tested with sea urchin embryos.

compounds. Once porewater was treated with these different columns, sea urchin embryos were incubated for 56 hours to early stage pluteus, then scored for mortality, arrested development or deformaties in the pluteus to determine which column treatment reduced toxicity.

Each treatment removed some amount of toxicity from each sample, though only at Olawalu was all toxicity removed, although there was less than 10% shown in the original porewater test. The most dramatic reductions in toxicity were with the C18 column treatments which removed most of the toxicity associated with samples from Kalaepohaku pier. Similar reductions with C18 were seen at other locations. The Verify AX column removed toxicity from Lipoa Place and WNA 1-3 at Kahekili and most at Kalaepohaku, however was only marginally effective in removing the toxicants from the north Kapalua Bay sample. There was not enough porewater remaining from the other sites for this treatment.

Porewater Toxicity Assays using Coral Calicoblast Cells from *Porites divaricata*

Corals were obtained from U.S. NOAA NOS CCEHBR's Coral Culture and Collaborative Research Facility in Charleston, SC and cultured at Haereticus for over four months.

Coral cells were isolated using a method described in Downs *et al.* (2010). Cells were incubated in cell culture media for 8-hours, and then placed in 250 microliters of "corrected porewater." On average, about 250,000 cells were added to each microplate well. Cells were incubated for eight hours, and then counted using a dye-exclusion viability stain (Napthol Blue Black). There was insufficient porewaters to run a TIE using isolated coral cells.

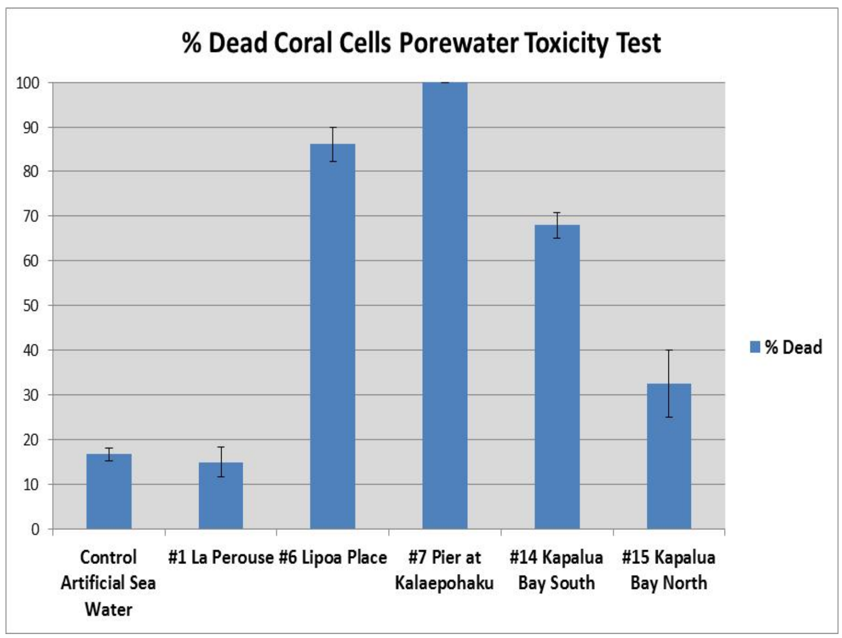


Figure 3 Porewater toxicity test using coral calicoblast cells. Porewater from 5 of the sampled sites were tested for toxicity against *Porites divaricata* calicoblast cells. Toxicity is expressed as percent mortality for the assay endpoint.

As shown in Fig. 4, Lipoa Place, Kalaepohalu and the south Bay of Kapalua showed the highest toxicity in the coral cell toxicity test of the sites tested. La Perouse, a long-term reference site was not different from the artificial seawater control. Of interest is the difference in toxicity between the north and south side of Kapalua Bay using the coral cell toxicity assay (Fig. 4).

Summary of Toxicity Testing

- 16 out of the 17 sites tested were positive for toxic-associated pollution; La Perouse, a long-term reference site was the only site not significantly different from the artificial sea water control
- Organic derived pollutants (as determined by the TIE) were predominate in most of the porewater samples. Toxicity still remained following removal of these organics for three sites, Lipoa Place, Kapalua Bay, and Kalaepohaku. The latter results suggest the possibility of metal toxicity.
- Setbacks to this study were the inability to get large samples of porewater from a single collection point. A single porewater assay, requires 8.5 mL of porewater. Over 15 mL of porewater is required to run a single TIE treatment. We are unable to obtain enough porewater to run a six-treatment TIE (standard). Due to samples being collected and shipped off island for analysis, it was not possible to judge the actual amount of porewaters contained in the Teflon bags. The amount that was extracted from the sediments was approximately 25 ml. It is suspected that because the samples were from sand areas adjacent immediately to coral reefs or coral colonies, the amount of porewater was less than expected in general for the volume of sediment collected. It was not financially feasible to acquire additional samples. Year 2 of this project will focus on ensuring sufficient volumes of porewater samples are available for testing.
- The solid phase columns used in this study, and another similar study, were shown to be effective in directly identifying the nature of toxicant as demonstrated with several samples the complete removal of toxicity after treatment of the porewater with various column matrices.

Preliminary Toxicity Identification Evaluation, Phase II

To obtain preliminary data for Year 2 activities, SPE columns used to treat porewater in three of the TIE samples, SSB3, South of the South Seep, and Kapalua North, were sent to Jupiter Labs (Jupiter, FL) for contaminant chemistry screening. (Note: This is preliminary information in support of Yr. 2 activities that was possible as additional data in Yr. 1)

Sample 1- SSB3:
Hypersep C-18
Verify AX
Strata AN

Sample 2 - Kapalua North:
Bond Elute C-18
Verify AX

Sample 3-South, South Seep:
Bond Elute C-18
Verify AX

The samples were tested for organophosphate pesticides (OP), herbicides and pharmaceutical and personal care products (PPCP), steroidal compounds and estrogenic mimics. For OPs, herbicides and PPCPs, the samples were extracted with 5ml of MeOH under aggressive vacuum, filtered and then 500 µL was added to mobile phase A (UP H_20 Ammonium Acetate / Formic Acid) to insure chromatographic peak shape. For steroidal compounds and estrogenic mimics, the samples were treated under pH conditions to increase Mass Spectrometry source ionization during the HPLC/MS/MS analysis. All samples were run using the AB SCIEX 5500 Q-Trap with blanks and controls to insure the quality of the data. Detection of a compound is denoted with an 'X' in Table 1.

Table 1 Summary of chemical findings for 3 sites in West Maui.

Analyte	SSB3 Strata AN	SSB3 Hypersep C18	SSB3 Verify AX	Kapalua Verify AX	Kapalua Bond Elute C18	SEEP Bond Elute C18	SEEP Verify AX
Caffeine	X						
Carbamazepine	X	X	X			X	X
Primidone	X	X	X				
Sulfamethoxazole	X	X	X				
PCF					X		
1-bromo 2 nitro benzene			X				
Atrazine		X			X	X	
Malathion					X		
Simazine						X	

Microbiology

Introduction

The Clean Water Act (CWA) of 1972 establishes the regulatory framework for controlling pollutant discharges into US waters and establishes water quality standards for surface waters and has been amended in 1977 (Clean Water Act of 1977) and 1987 (Water Quality Act of 1987). The Safe Drinking Water Act of 1974 (SDWA, with amendments in 1986 & 1996) also regulates water quality from chemical and biological pollutants. The EPA approves sampling procedures and analytical methods used to determine chemical, microbiological and radiological components in wastewater under the CWA as well as methods for drinking water contaminants under the SDWA. These regulations also extend to marine and freshwater recreational waters and with associated biological water quality criteria.

Bacterial Water Quality Standards for Recreational Waters (Fresh and Marine waters) is assessed by fecal bacteria as an indicator of the possible presence of pathogens in surface waters and the risk of disease and are used as sewage indicators for regulating water quality and beach closures. Though these standards are directed at human health safety, there is growing evidence that contaminated waters from sewage can carry pathogens that affect marine organisms. In the Florida Keys, fecal contamination has been traced to coral reefs (Lipp et al. 2002; Lipp & Griffin 2004) and found in coral mucus. *Serratia marcescens* has been shown as a causative agent to disease in *Acropora palmata* (White Pox; acropora serratiosis) (Patterson *et al*. 2002; Sutherland 2003; Sutherland *et al*. 2010).

Prior to 1986 bacterial water quality testing was based on fecal coliforms with recommended maximum densities not to exceed geometric means of 200 organisms per 100 ml in recreational waters. In 1986 the criteria was updated and the primary indicators of fecal contamination included fecal coliforms, enterococci and *Escherichia coli*. Epidemiological studies found that enterococci and *E. coli* had a higher degree of association with outbreaks of certain diseases than the fecal coliforms. Thus enterococci (criteria 33/100ml freshwater; 35/100ml marine waters) and *E. coli* (126/100 ml freshwater) were recommended as the indicator bacteria for in the 1986 *Ambient Water Quality Criteria for Bacteria* (EPA 2003). The Beaches Environmental Assessment and Coastal Health (BEACH) Act of 2000 required each state and territory with coastal recreational waters to adopt into their water quality standards by 2004 bacteria criteria that are as protective of human health as the those proposed in 1986 (EPA 2004). In 2012, EPA released new criteria for recreational waters (EPA 2012) that provides two sets of threshold concentrations. It consists of three components: magnitude, duration and frequency (EPA 2012a). As science evolves, new indicators and methods are being evaluated and updated by EPA. The most recent addition is molecular testing using quantative real-time polymerase chain reaction (qPCR) for enterococci (EPA Method 1611; EPA 2012b).

Hawaii Water Quality Standards are set forth in their public law HAR §11-54. HAR §11-54-3(c) and define three waterbody types (embayment, open coastal and oceanic) and uses a tiered classification scheme of “AA” and “A” (HIDOH 2012). Hawaii’s bacteria water quality criteria uses enterococci as a primary bacterial indicator and limits are set at a geometric mean of 35 colony-forming units (CFU)/100mL and a single sample maximum limit of 104 CFU/100mL. Hawaii and other monitoring programs across the US have found this indicator to be problematic. This finding is also supported by several studies showing that in tropical environments this bacteria can multiply outside of the human body (Byappanahalli & Fujioka 2004) and can also be found in feces of various wildlife species (e.g., feral mammals and birds). Although not specified by the EPA as an indicator species, *Clostridium perfringens* was identified as an effective tracer of fecal contamination and is used by HIDOH as a secondary tracer to help confirm high bacterial counts when enterococci levels are exceeded.

For this study, however, *Clostridium perfringens* density determinations were not determined due to the lack of available facilities having proper biocontainment. This organism is a Gram-positive anaerobe which produces spores and requires anaerobic culturing conditions. In addition to causing potentially fatal food poisoning, it is also causes gas gangrene. Culture of this organism requires a Biological Safety Laboratory, Level 2 since it is pathogenic, produces several toxins, and has a thick cell wall which makes it much more difficult to kill as compared to Gram negative bacteria (such *as E. coli* or Vibrios). Since it produces spores, containment of potentially harmful particles is problematic.

Two additional pathogenic bacterial were also included in this study. Total *Staphylococcus and Staphylococcus aureus,* known human pathogens that can be found in marine waters, were also enumerated; in addition, these bacteria are of interest because of concern over some strains having multiple antibiotic resistance (MRSA). Further, Maui has a reported high incidence of MRSA related infections, exceeding those of the US Mainland almost two-fold (HHIC 2006). Levels of *Serratia marcescens* were also tested because it is associated with sewage and a potential human pathogen. In addition, *S. marcescens* is associated with a Caribbean coral disease, acroporid serratiosis (aka white pox disease).

Microbial Aspects of West Maui Coastal Waters

Evaluation of the microbial aspects of the coastal waters of West Maui is objective 3 of Year 1 of this study. Specific questions addressed as part of this objective are as follows:

1. What are the levels of fecal indicator bacteria in waters of Maui, HI in dry versus wet seasons?
2. Do fecal indicator bacteria densities exceed water quality standards (location versus time)?
3. What are levels of the sewage-associated and potential human and coral pathogen, *Serratia marcescens,* in coastal waters of Maui?

4. What are the levels of the human pathogen, *Staphylococcus aureus*, and total *Staphylococcus* in coastal waters of Maui in dry versus wet seasons?
5. Do indicator bacteria densities change based on the tidal stage of sample collection?
6. Are *Staphylococcus* associated with coral mucus?

YEAR 1 SITES SURVEYED FOR BACTERIAL INDICATORS ANALYSIS

A total of 18 sites were sampled in Year 1 of this study for bacterial indicators as depicted on the map with yellow arrows in Fig. 5.

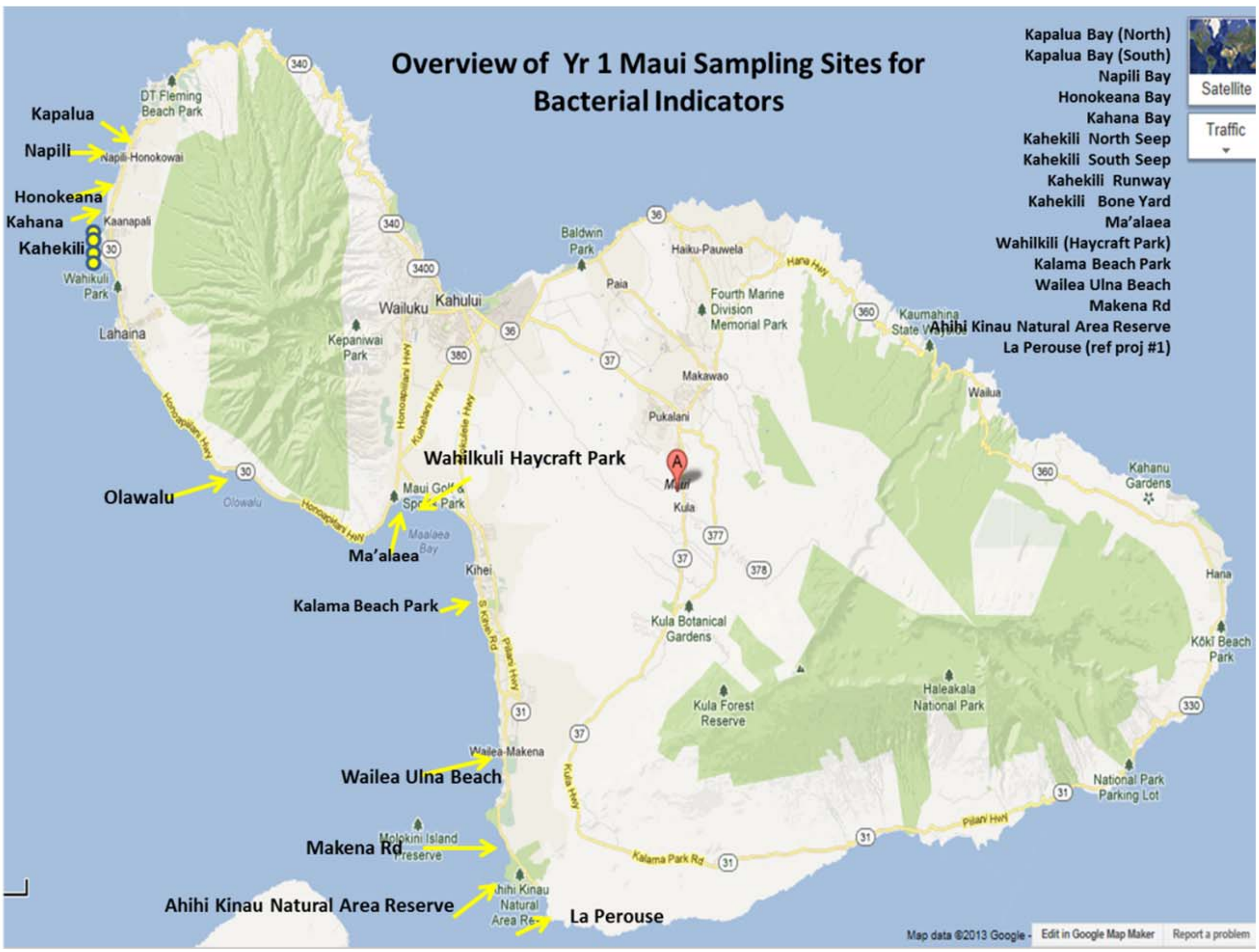


Figure 4 Map depicting Year 1 sampling sites along the Maui coast for bacterial indicators.

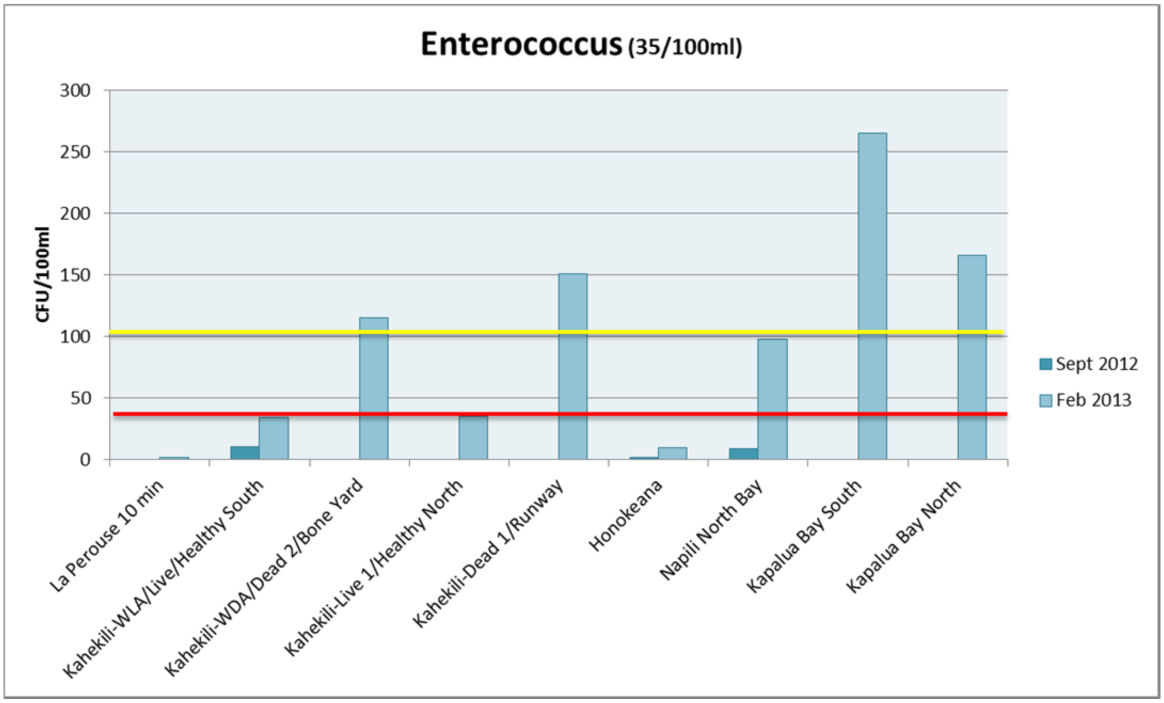
Table 2 List of Maui sites sampled during project Year 1.

Sites in Project	Sample Type	Microbiology Sample collected 9/12	Microbiology Sample collected 2/13
La Perouse (Reference site)	Tox 1/ Micro	x	x
Makena Road 7381	Tox 2	x	
Makena Reserve (little beach area in bend)	Micro		x
Wailea, Ulna Beach Park	Tox 5/ Micro	x	
Kalama Beach Park		x	
Olawalu East	Tox 8 Micro		x
Olawalu West	Tox 9 Micro		x
Kahekili WLA/Live2 -Westin Live zone A also Kahekili Healthy South	Micro	x	x
Kahekili Sand Channel	Micro		x
Kahekili WDA/Dead2 - Westin Dead Zone A also Kahekili Bone Yard	Micro	x	x
Kahekili SS- South Seep 1-3	Tox/ Micro	x	
Live 1 ALSO Kahekili Healthy North	Micro	x	x
DEAD 1 ALSO Kahekili Runway/Dead 1	Micro	x	x
Honokeana Bay	Tox 12	x	x
Napili Bay North	Tox 13/ Micro	x	x
Napili Bay South	Micro		x
Kapalua Bay South	Tox 14 Micro	x	x
Kapalua Bay North	Tox 15 Micro	x	x

Figure 5 Kahekili sites sampled in Sept 2012 and Feb 2013 showing co-location of sites with different names.

1. What are the levels of fecal indicator bacteria in waters of Maui, HI in dry vs. wet seasons?

Nine of the 18 sites were sampled in both dry (9/12) and wet (2/13) seasons: La Perouse, Westin Live Zone A (WLA/Live2) AKA Kahekili Bone Yard, Westin Live Zone A (WLA/Live2) AKA Kahekili Healthy South, Live 1 AKA Kahekili Healthy North, Dead 1 AKA Kahekili Runway, Napili Bay North, Kapalua Bay South and Kapalua Bay North (photos pg 4-10). Levels of fecal indicator bacteria in dry (Sept) vs wet (Feb) seasons are shown in Figure 7.



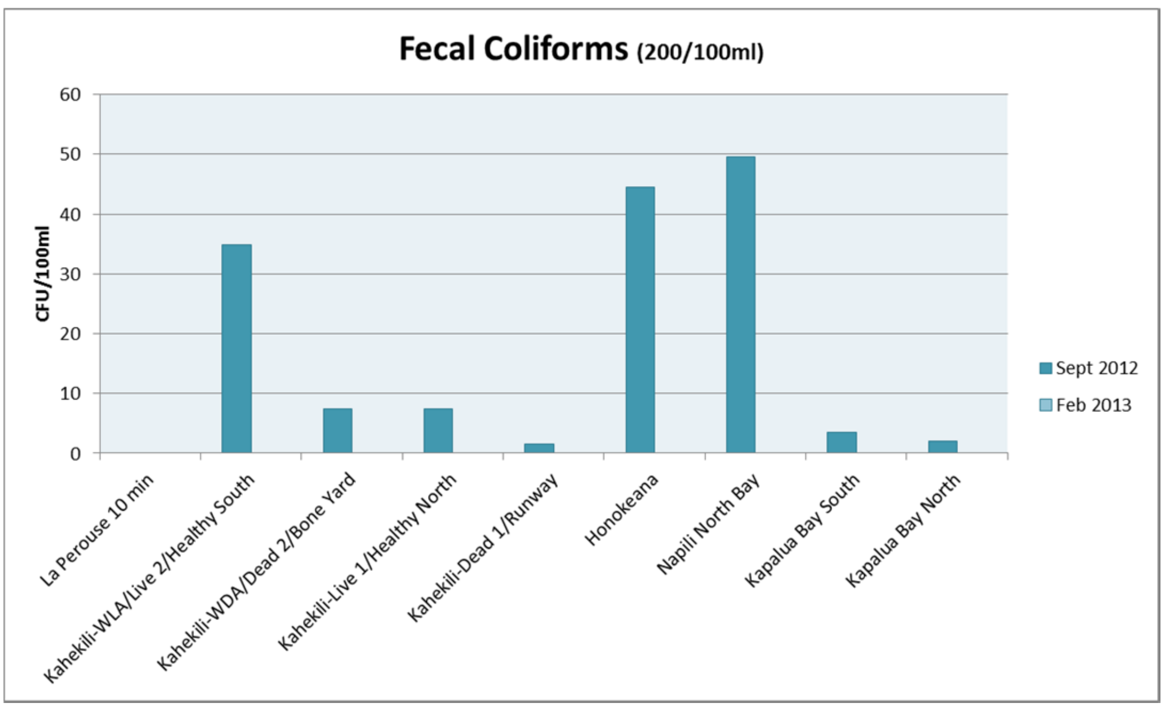


Figure 6 Levels of Indicator Bacteria in Dry (Sept) vs Wet (Feb) Seasons. Red line indicates the level of Enterococcus triggering a 5 day follow-up sampling to determine geometric mean of the bacterial levels. Yellow line is the single sample maximum limit for Enterococcus in marine waters that trigger action by public health officials.

2. Do fecal indicator bacteria densities exceed water quality standards (location vs time)?

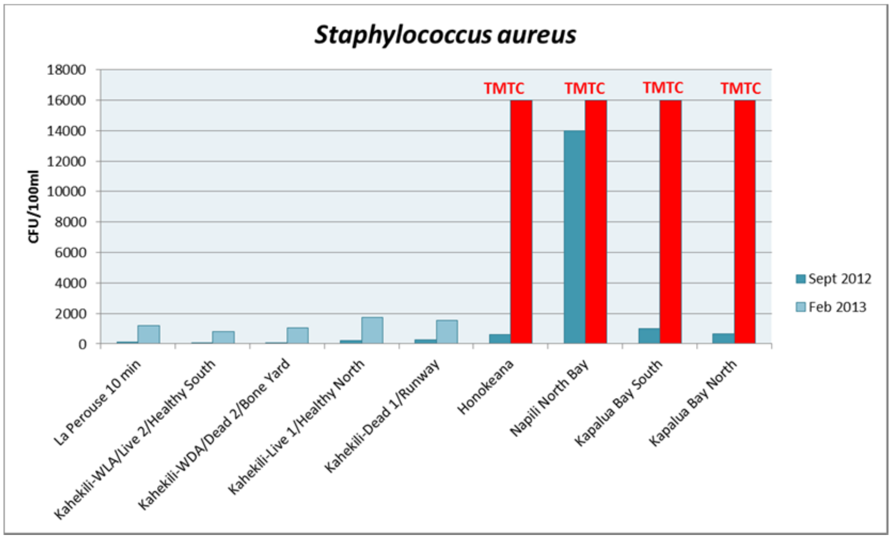
There were no sites that exceeded the water quality standards in September 2012, however in February 2013, three sites achieved action levels (35cfu/100ml) that would indicate the need for follow-up sampling to determine the geometric mean (Fig. 7). Four sites (Kihekili-Bone Yard, Kihekili-Runway, Kapalua North Bay and Kapalua South Bay) exceeded the single limit level of 104cfu/100ml, with a fifth site (Honokeana) slightly below at 97.5cfu/100ml.

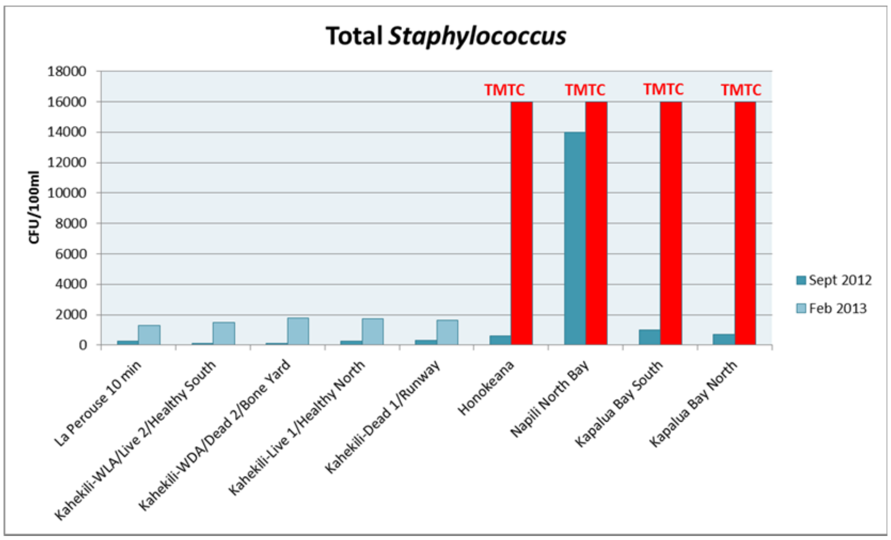
3. What are levels of the sewage-associated and potential human and coral pathogen, *Serratia marcescens,* in coastal waters of Maui?

Serratia marcescens is not a water quality bacterial indicator, though it has been shown as a human pathogen associated with sewage. These bacteria have been associated with coral disease in the Caribbean, but there are no reports of an association with Pacific corals, possibly because of the intensity of investigation in the Caribbean versus Pacific with sewage-related risk factors for coral disease. Two locations in Kahekili showed levels above those of most other sites surveyed, except Napili Bay (north) (Fig. 8). No coral samples were examined for health status or levels of *S. marcescens* as part of this study. The intent of sampling for these bacteria was to determine bacterial levels to determine if there was sufficient data to warrant a more targeted study of whether a link may exist with coral disease incidence. Data on coral disease incidence and location from Hawaii DLNR and NOAA coral disease surveys will be used to determine if a correlation may exist.

4. What are the levels of the human pathogen, *Staphylococcus aureus*, and total *Staphylococcus* in coastal waters of Maui in dry vs wet seasons?

There are no water quality standards for *Staphylococcus spp.* and little information for levels normally found in marine waters. Figure 8 shows levels of *Staphylococcus aureus* and Total *Staphylococcus* at nine locations along the Maui coastline and provides a comparison of levels found in dry versus wet seasons. Levels were obviously elevated during the wet season. In February 2013, even 1ml of the water samples from the northwestern locations yielded bacterial levels that completely over-grew the culture plates and are designated as the red columns labeled TMTC (too many to count).





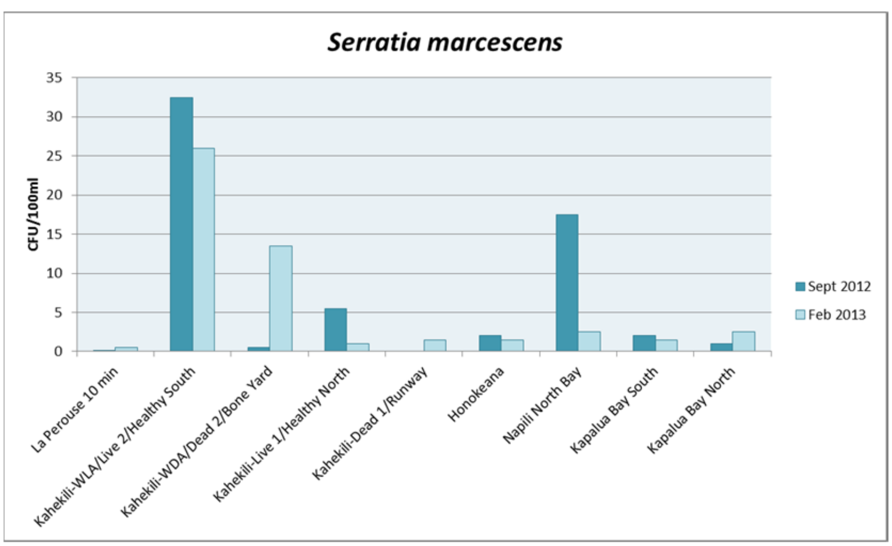


Figure 7 Levels of Potential Pathogenic Bacteria in Dry (Sept) vs Wet (Feb) Seasons. Red columns labeled TMTC indicates too many bacterial colonies to reliably count.

5. Do indicator bacteria densities change based on the tidal stage of sample collection?

Water samples were collected at different tidal stages to determine if levels of fecal coliforms or *Staphylococcus* differed with the tide. To test this, water samples were collected at the southern side of Napili Bay on January 27, 2012, beginning at 6:30am with the ebbing tide (2 hr after high tide), then low tide (10:30am), flood tide (12:30pm), flood tide (2:30pm), high tide (4:30pm), ebbing tide (6:30pm) and low tide (10:30pm) (Table 3). These data indicate that levels of *Staphylococcus* are constant during daylight hours regardless of the tidal stage, however in the dark levels are slightly increased. Follow-up sampling would be required to determine whether levels change significantly in dark vs light conditions. Fecal coliforms however do seem to fluctuate with tidal cycle. It is interesting to note that this same location was re-sampled on January 31, 2013 at 10am and fecal coliforms were 25cfu/100ml and enterococcus was undetectable while across the bay on the north side of Napili, fecal coliforms were 7.5cfu/100ml and enterococcus was 97.5cfu/100ml (Fig 7).

Table 3 The Effect of Tidal Stage on Bacterial Levels.

TIME	TIDE	FECAL COLIFORMS CFU/100ML	STAPHYLOCOCCUS CFU/100ML
6:30AM	EBBING	1.5	3450
10:30AM	LOW	11.5	3000
12:30PM	FLOOD	0	1700
2:30PM	FLOOD	4	3350
4:30PM	HIGH	15.5	3150
6:30PM	EBBING	2.5	3600
10:30PM	LOW	14.5	4500

6. Are Staphylococcus associated with coral mucus?

Preliminary data from the February 2013 sampling was inconclusive because the lowest dilution tested of coral mucus overgrew the plates. However it was clear that the water column levels were less concentrated with *Staphylococcus* than coral mucus since water samples did yield countable colony forming units. This question is being further tested in June 2013. Questions being posed are:

What are the levels of Staphylococcus in the water column vs coral mucus?
Do levels of Staphylococcus follow a gradient in respect to coral colony?
Do levels of Staphylococcus differ between disease vs healthy conditions of a species?
Do levels of Staphylococcus differ among coral species?

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APPENDIX I – SEA URCHIN POREWATER TOXICITY TEST DATA

Sample ID	# of embryos	# arrested	# deformed	# dead
#1 La Perouse				
1	22	0	0	0
2	28	0	0	0
3	33	0	0	1
4	26	0	0	0
#2 Makena Road				
1	23	0	1	0
2	34	0	2	0
3	24	0	1	1
4	25	0	2	0
#3 Makena Park, Little beach				
1	25	1	2	0
2	26	1	1	1
3	31	1	1	1
4	22	0	0	0
#4 Wailea, Polo Beach				
1	26	0	1	0
2	28	0	2	0
3	30	0	0	0
4	30	0	3	1
#5 Ulua Beach Park				
1	35	0	0	0
2	28	0	3	0
3	21	0	1	0
4	24	0	3	1
#6 Lipoa Place				
1	36	5	8	5
2	38	7	8	5
3	40	6	7	7
4	23	6	8	7
#7, pier at Kalaepohaku				
1	22	16	0	6
2	18	16	0	2
3	28	22	2	4
4	30	16	4	10
#8, Olawalu East				
1	27	0	6	0
2	29	0	4	0
3	34	0	6	2
4	30	0	5	0
#9, Olawalu West				
1	22	0	6	2
2	41	0	4	3
3	28	0	6	3
4	29	0	5	1

Sample ID	# of embryos	# arrested	# deformed	# dead
#10, Lahaina				
1	17	0	0	17
2	25	0	0	25
3	28	0	0	28
4	31	0	0	31
#11, Kahana Bay				
1	27	4	9	0
2	27	8	11	0
3	34	7	6	0
4	28	7	5	0
#12, Honokeana Bay				
1	30	0	15	15
2	29	0	11	18
3	43	0	23	20
4	25	0	15	10
#13, Napili Bay				
1	26	5	2	1
2	18	8	4	1
3	24	4	4	0
4	27	9	5	2
#14, Kapalua Bay South				
1	36	14	16	6
2	29	12	12	5
3	21	13	5	3
4	19	8	6	5
#15, Kapalua Bay North				
1	29	8	16	5
2	20	2	15	3
3	20	2	14	4
4	25	3	18	2
WNA 1-3				
1	18	0	1	0
2	29	0	1	0
3	28	0	0	0
4	34	0	2	0
South of the South Seep 1-3				
1	41	0	3	1
2	22	0	1	0
3	18	0	0	0
4	19	0	2	0
Site near North Plume				
1	35	0	4	1
2	19	0	3	2
3	26	0	0	3
4	22	0	3	2

Appendix II Coral Cell Porewater Toxicity Test Results

	Total cells at beginning			live cells		Dead cells
artificial sea water						
1	18	**225000**	14	**175000**	**3**	**37500**
2	20	**250000**	16	**200000**	**3**	**37500**
3	21	**262500**	16	**200000**	**4**	**50000**
4	17	**212500**	13	**162500**	**2**	**25000**
#1 La Perouse		**0**		**0**		**0**
1	16	**200000**	14	**175000**	**3**	**37500**
2	19	**237500**	15	**187500**	**4**	**50000**
3	24	**300000**	21	**262500**	**4**	**50000**
4	20	**250000**	18	**225000**	**1**	**12500**
#6 Lipoa Place		**0**		**0**		**0**
1	9	**112500**	2	**25000**	**6**	**75000**
2	15	**187500**	1	**12500**	**13**	**162500**
3	19	**237500**	2	**25000**	**15**	**187500**
4	17	**212500**	2	**25000**	**15**	**187500**
#7, pier at Kalaepohaku		**0**		**0**		**0**
1	22	**275000**	0	**0**	**15**	**187500**
2	24	**300000**	0	**0**	**17**	**212500**
3	20	**250000**	0	**0**	**17**	**212500**
4	28	**350000**	0	**0**	**20**	**250000**
#14, Kapalua Bay South		**0**		**0**		**0**
1	19	**237500**	4	**50000**	**9**	**112500**
2	24	**300000**	5	**62500**	**14**	**175000**
3	25	**312500**	8	**100000**	**12**	**150000**
4	16	**200000**	4	**50000**	**9**	**112500**
#15, Kapalua Bay North		**0**		**0**		**0**
1	15	**187500**	8	**100000**	**8**	**100000**
2	15	**187500**	10	**125000**	**6**	**75000**
3	22	**275000**	15	**187500**	**6**	**75000**
4	23	**287500**	18	**225000**	**3**	**37500**

APPENDIX III – SEA URCHIN TOXICITY IDENTIFICATION EVALUATION RESULTS

#6 Lipoa Place				
Porewater Test	# of embryos	# arrested	# deformed	# dead
1	36	5	8	5
2	38	7	8	5
3	40	6	7	7
4	23	6	8	7
TIE-C18	# of embryos	# arrested	# deformed	# dead
1	24	4	9	0
2	25	6	6	0
3	30	5	11	0
TIE-Verify AX	# of embryos	# arrested	# deformed	# dead
1	22	0	0	0
2	15	0	0	0
3	19	0	0	0

#7, Pier at Kalaepohaku	# of embryos	# arrested	# deformed	# dead
Porewater Test				
1	22	16	0	6
2	18	16	0	2
3	28	22	2	4
4	30	16	4	10
TIE-C18	# of embryos	# arrested	# deformed	# dead
1	17	2	0	0
2	19	1	0	0
3	26	3	0	0
TIE-Verify AX	# of embryos	# arrested	# deformed	# dead
1	15	0	0	1
2	18	0	0	0
3	20	1	0	0

#8, Olawalu East				
Porewater Test	# of embryos	# arrested	# deformed	# dead
1	27	0	6	0
2	29	0	4	0
3	34	0	6	2
4	30	0	5	0
TIE-C18	# of embryos	# arrested	# deformed	# dead
	30	0	6	0

#9, Olawalu West				
Porewater Test	# of embryos	# arrested	# deformed	# dead
1	22	0	6	2
2	41	0	4	3
3	28	0	6	3
4	29	0	5	1
TIE-C18	# of embryos	# arrested	# deformed	# dead
	28	0	0	0

#12, Honokeana Bay				
Porewater Test	# of embryos	# arrested	# deformed	# dead
1	30	0	15	15
2	29	0	11	18
3	43	0	23	20
4	25	0	15	10
TIE-C18	# of embryos	# arrested	# deformed	# dead
	25	0	5	0
	30	0	6	0
	35	0	8	2

#15, Kapalua Bay North				
Porewater Test	# of embryos	# arrested	# deformed	# dead
1	29	8	16	5
2	20	2	15	3
3	20	2	14	4
4	25	3	18	2
TIE-C18	# of embryos	# arrested	# deformed	# dead
1	31	0	4	0
2	43	0	8	1
3	30	0	5	0
TIE-Verify AX	# of embryos	# arrested	# deformed	# dead
1	47	0	19	0
2	35	0	14	0
3	27	0	20	0

#16 Site near North Plume				
Porewater Test	# of embryos	# arrested	# deformed	# dead
1	35	0	4	1
2	19	0	3	2
3	26	0	0	3
4	22	0	3	2
TIE-C18	# of embryos	# arrested	# deformed	# dead
	15	0	0	0
	19	0	0	0
	22	0	0	2
TIE-Verify AX	# of embryos	# arrested	# deformed	# dead
	16	0	0	0
	25	0	0	0
	23	0	0	0

Appendix IV – Preliminary Toxicity Identification Evaluation Phase II

Table 1. Findings from TIE fractionation and analytical chemistry.

Analyte	ssb3 strata	ssb3 c18	ssb3 ax	Kapalua AX	Kapalua C18	scep c18	scep ax
Acetaminophen 1							
Caffeine 1	x						
carbamazepine 1	x	x	x			x	x
ciprofloxacin hcl 1							
diclofenac sodium salt 1							
erythromycin 1							
fluoxetine 1							
primidone 1	x	x	x				
progestrone 1							
sulfamethoxazole 1	x	x	x				
testosterone 1							
Trimethoprim 1							
2,4,5T 1							
2,4,5TP 1							
2.4-D 1							
2.4-DB 1							
3,5-dichlorobenzoic acid 1							
4 nitrophenol 1							
Acifluorfen 1							
Bentazon 1							
dalapon 1							
Dicamba 1							
dichloroprop 1							
Dinoseb 1							
MCPA 1							
Mecoprop-P 1							
PCF 1					x		
picloram 1							
p tert amyl phenol 1							
4 tert butyllphenol 1							
bis a 1							
ESTRONE 1							
Nonylphenol 1							
4 tert octylphenol 1							
17 a ethynylestradiol 1							
17 b Estradiol 1							
1 bromo 2 nitro benzene 1			x				
aspon 1							
Atrazine 1		x			x	x	
azinophos methyl Guthion 1							
azinophos-ethyl 1							
carbophenothion 1							
chlorfenvinphos 1							
chlorpyriphos 1							
chlorpyriphos- methyl 1							
crotoxyphos 1							
cumaphos 1							
diazinon 1							
dichlorofenthion 1							
dichlorvos 1							
dichrotophos 1							
dimethoate 1							
dioxathion 1							
disulfoton 1							
epn 1							
ethion 1							
ethoprop 1							
ethyl parathion 1							
famphur 1							
fenchlorphos ronnel 1							
fenitrothion 1							
fensulfothion 1							
fenthion 1							
fonophos 1							
leptophos 1							
malathion 1					x		
merphos 1							
m parathion 1							
mevinphos 1							
monocrotophos 1							
naled 1							
phorate 1							
phosmadion 1							
phosmet 1							
Simazine 1						x	
sulfotepp 1							
sulprofos Bolstar 1							
Teep 1							
terbufos 1							
tetrachlorvinphos 1							
thionazin 1							
tokuthion 1							
trichlorfon 1							
trichloronate 1							

"X" indicates presence of contaminant

SPE columns for three samples, SSB3, South of the South Seep, and Kapalua North, that reduced toxicity (i.e., binding toxicant) were analyzed by Jupiter Labs for contaminant chemistry screening. The samples arrived frozen and in good condition and were stored below zero until extraction.

All samples we run using the AB SCIEX 5500 Q-Trap with blanks and controls to insure the quality of the data.

Edward J. Dabrea | Principal | www.jupiterlabs.com

Office: 561.575.0030
Mobile: 561.262.8737
Email: edabrea@jupiterlabs.cc

Jupiter
Environmental Laboratories, Inc.

Appendix V. Sampling Site Coordinates

September 2013 Sampling Sites	Lat	Long
WLA/Live2	20°56'213.324"N	156° 41' 37.006" W
WLB	20° 56' 13.422" N	156° 41' 37.039" W
WDA/Dead2	20° 56' 14.174" N	156° 41' 37.374" W
WDB	20° 56' 14.176" N	156° 41' 37.477" W
Dead1	20° 56' 22.134" N	156° 41' 37.009" W
Live1	20° 56' 20.799" N	156° 41' 36.892" W
WSA	20° 56' 16.817" N	156° 41' 35.672" W
WSB	20° 56' 15.321" N	156° 41' 35.661" W
La Perouse	20° 35' 26.459" N	156° 24' 46.744" W
Makena Road	20° 37' 17.653" N	156° 26' 22.092" W
WNA, Kahekili	20° 56' 27.540"N	156° 41' 33.480"W
North Seep, Kahekili	20° 56' 24.690"N	156° 41' 34.080"W
Maalaea	20° 47' 31.518" N	156° 30' 35.924"W
Waipuilaini	20° 45' 17.310" N	156° 27' 35.038"W
Kalama Park	20° 43' 45.176" N	156° 27' 07.795" W
January/February 2013 Sampling Sites	**Lat**	**Long**
La Perouse	20°35'45.67"N	156°24'57.31"W
Makena	20°37'3.47"N	156°26'18.13"W
CRAMP Kayak (Olowalu)	20°48'32.47"N	156°36'48.71"W
CRAMP South (Olowalu)	20°48'32.80"N	156°36'40.79"W
Kapalua North	21° 0'1.33"N	156°40'1.16"W
Kapalua South	20°59'56.22"N	156°40'3.25"W
Napili North	20°59'47.72"N	156°40'0.19"W
Napili South	20°59'40.70"N	156°40'2.93"W
Honokeana	20°59'32.24"N	156°40'8.04"W
Runway	20°56'22.34"N	156°41'36.85"W
Healthy North	20°56'20.72"N	156°41'37.25"W
Bone Yard	20°56'14.42"N	156°41'36.53"W
Healthy South	20°56'12.92"N	156°41'36.50"W
Sand Channel	20°56'10.46"N	156°41'36.10"W

Appendix VI. Summarized Data from September 2012 Water Quality Sampling.

ENTEROCOCCUS (35 cfu/100ml)	A	B	mean	SE
Westin Live Coral (9/18/12)	11.0	10.8	10.9	0.1
Westin Dead Zone (9/18/12)	0.0	0.0	0.0	0
Westin South Seep (9/18/12)	0.0	0.0	0.0	0
La Perouse- 30 min (9/19/12)	0.0	0.0	0.0	0
7381 McKenna Rd (9/19/12)	0.0	0.0	0.0	0
Kapalua (1st sample)	9.6	9.2	9.4	0.2
Kalama Beach Park	0.0	0.0	0.0	0
Wailea	0.0	0.0	0.0	0
Kahekili Live 1 Coral class	0.0	0.0	0.0	0
Kahekili Live 2 Coral class	0.0	0.0	0.0	0
Kahekili Dead 1 Zone class	0.0	0.0	0.0	0
Kahekili Dead 2 Zone class	0.0	0.0	0.0	0
Kahekili South Seep class	0.0	0.0	0.0	0
Ma'alaea class	0.0	0.0	0.0	0
Honokena class	0.0	3.0	1.5	1.5
Wahilkuli class	1.0	0.0	0.5	0.5
Napili	11.0	7.0	9.0	2
Kapalua 1 north	0.0	0.0	0.0	0
Kapalua 2 south	0.0	0.0	0.0	0
(FECAL COLIFORMS (200cfu/ 100ml)				
Westin Live Coral	31.0	38.6	34.8	3.8
Westin Dead Zone	1.2	1.2	1.2	0
Westin South Seep	0.0	0.0	0.0	0
La Perouse- 30 min	0.8	3.6	2.2	1.4
7381 McKenna Rd	0.4	0.8	0.6	0.2
Kapalua	31.8	22.4	27.1	4.7
La Perouse parking (sterile)	ND			
La Perouse- 10 min	ND			
7381 McKenna Rd	ND			
Kalama Beach Park	2.0	1.0	1.5	0.5
Wailea	1.0	0.0	0.5	0.5
Kahekili Live 1 Coral class	7.0	8.0	7.5	0.5

Kahekili Live 2 Coral class	**2.0**	**6.0**	**4.0**	**2**
Kahekili Dead 1 Zone class	**1.0**	**2.0**	**1.5**	**0.5**
Kahekili Dead 2 Zone class	**7.0**	**8.0**	**7.5**	**0.5**
Kahekili South Seep class	**0.0**	**0.0**	**0.0**	**0**
Ma'alaea class	**0.0**	**4.0**	**2.0**	**2**
Honokena class	**26.0**	**63.0**	**44.5**	**18.5**
Wahilkuli class	**15.0**	**3.0**	**9.0**	**6**
Napili	**26.0**	**73.0**	**49.5**	**23.5**
Kapalua 1 north	**1.0**	**3.0**	**2.0**	**1**
Kapalua 2 south	**3.0**	**4.0**	**3.5**	**0.5**
ESCHERICHIA COLI				
Westin Live Coral	**0.0**	**0.0**	**0.0**	**0**
Westin Dead Zone	**0.0**	**0.0**	**0.0**	**0**
Westin South Seep	**0.0**	**0.0**	**0.0**	**0**
La Perouse- 30 min	**0.2**	**0.6**	**0.4**	**0.2**
7381 McKenna Rd	**0.0**	**0.0**	**0.0**	**1.6**
Kapalua	**25.4**	**22.2**	**23.8**	
La Perouse parking (sterile)	**ND**			
La Perouse- 10 min	**ND**			
7381 McKenna Rd	**ND**			
Kalama Beach Park	**0.0**	**0.0**	**0.0**	**0**
Wailea (Ulna Beach)	**0.0**	**0.0**	**0.0**	**0**
Kahekili Live 1 Coral class	**0.0**	**0.0**	**0.0**	**0**
Kahekili Live 2 Coral class	**0.0**	**0.0**	**0.0**	**0**
Kahekili Dead 1 Zone class	**0.0**	**0.0**	**0.0**	**0**
Kahekili Dead 2 Zone class	**2.0**	**6.0**	**4.0**	**2**
Kahekili South Seep class	**0.0**	**0.0**	**0.0**	**0**
Ma'alaea class	**0.0**	**0.0**	**0.0**	**0**
Honokena class	**1.0**	**0.0**	**0.5**	**0.5**
Wahilkuli class (Haycraft Park)	**4.0**	**0.0**	**2.0**	**2**
Napili	**2.0**	**2.0**	**2.0**	**0**
Kapalua 1 north	**1.0**	**0.0**	**0.5**	**0.5**
Kapalua 2 south	**0.0**	**0.0**	**0.0**	**0**

SERRATIA MARCESCENS				
Westin Live Coral	33.6	31.2	32.4	1.2
Westin Dead Zone	0.0	0.0	0.0	0
Westin South Seep	0.0	0.0	0.0	0
La Perouse- 30 min	0.2	0.0	0.1	0.1
7381 McKenna Rd	0.0	0.2	0.1	0.1
Kapalua	0.0	14.8	7.4	7.4
La Perouse parking (sterile)	ND			
La Perouse- 10 min	ND			
7381 McKenna Rd	ND			
Kalama Beach Park	0.0	0.0	0.0	0
Wailea	0.0	0.0	0.0	0
Kahekili Live 1 Coral class	6.0	5.0	5.5	0.5
Kahekili Live 2 Coral class	0.0	0.0	0.0	0
Kahekili Dead 1 Zone class	0.0	0.0	0.0	0
Kahekili Dead 2 Zone class	1.0	0.0	0.5	0.5
Kahekili South Seep class	0.0	0.0	0.0	0
Ma'alaea class	0.0	2.0	1.0	1
Honokena class	2.0	2.0	2.0	0
Wahilkuli class	2.0	1.0	1.5	0.5
Napili	19.0	16.0	17.5	1.5
Kapalua 1 north	2.0	0.0	1.0	1
Kapalua 2 south	2.0	2.0	2.0	0

STAPHYLOCOCCUS AUREUS				
Westin Live Coral (est TMTC)	TMTC	TMTC	TMTC	TMTC
Westin Dead Zone (est TMTC)	TMTC	TMTC	TMTC	TMTC
Westin South Seep	3.4	6.2	4.8	1.4
La Perouse- 30 min (est TMTC)	TMTC	TMTC	TMTC	TMTC
7381 McKenna Rd (est TMTC)	TMTC	TMTC	TMTC	TMTC
Kapalua (est TMTC)	TMTC	TMTC	TMTC	TMTC
La Perouse parking (sterile)	3500.0	2900.0	3200.0	300
La Perouse- 10 min	110.0	160.0	135.0	25
7381 McKenna Rd	550.0	530.0	540.0	10
Kalama Beach Park	300.0	490.0	395.0	95
Wailea	570.0	550.0	560.0	10

KAHEKILI LIVE 1 CORAL CLASS	210.0	190.0	200.0	10
KAHEKILI LIVE 2 CORAL CLASS	90.0	90.0	90.0	0
KAHEKILI DEAD 1 ZONE CLASS	340.0	170.0	255.0	85
KAHEKILI DEAD 2 ZONE CLASS	60.0	40.0	50.0	10
KAHEKILI SOUTH SEEP CLASS	43.0	64.0	53.5	10.5
MA'ALAEA CLASS	300.0	570.0	435.0	135
HONOKENA CLASS	600.0	600.0	600.0	0
WAHILKULI CLASS	180.0	140.0	160.0	20
NAPILI	14100.0	13900.0	14000.0	100
KAPALUA 1 NORTH	470.0	880.0	675.0	205
KAPALUA 2 SOUTH	1230.0	760.0	995.0	235
TOTAL STAPH				
WESTIN LIVE CORAL	TMTC	TMTC	TMTC	TMTC
WESTIN DEAD ZONE	TMTC	TMTC	TMTC	TMTC
WESTIN SOUTH SEEP	9.6	11.6	10.6	1
LA PEROUSE- 30 MIN	TMTC	TMTC	TMTC	TMTC
7381 MCKENNA RD	TMTC	TMTC	TMTC	TMTC
KAPALUA	TMTC	TMTC	TMTC	TMTC
LA PEROUSE PARKING (STERILE)	8200.0	6500.0	7350.0	850
LA PEROUSE- 10 MIN	230.0	290.0	260.0	30
7381 MCKENNA RD	112.0	120.0	116.0	4
KALAMA BEACH PARK	340.0	510.0	425.0	85
WAILEA	790.0	890.0	840.0	50
KAHEKILI LIVE 1 CORAL CLASS	270.0	250.0	260.0	10
KAHEKILI LIVE 2 CORAL CLASS	120.0	140.0	130.0	10
KAHEKILI DEAD 1 ZONE CLASS	370.0	220.0	295.0	75
KAHEKILI DEAD 2 ZONE CLASS	100.0	100.0	100.0	0
KAHEKILI SOUTH SEEP CLASS	43.0	64.0	53.5	10.5
MA'ALAEA CLASS	1380.0	950.0	1165.0	215
HONOKENA CLASS	600.0	610.0	605.0	5
WAHILKULI CLASS	190.0	140.0	165.0	25
NAPILI	14100.0	13900.0	14000.0	100
KAPALUA 1 NORTH	490.0	880.0	685.0	195
KAPALUA 2 SOUTH	1230.0	760.0	995.0	235

VII. Summary of Data from January/February Sampling

	Enterococcus (cfu/100 ml)	Fecal Coliforms (cfu/100 ml)	*Serratia marcescens* (cfu/100 ml)	Total *Staphylococcus* (cfu/100ml)
La Perouse	1.5	9	0.5	1290
Makena	29	1	0	1075
CRAMP Kayaks 1 (Olowalu)	8.5	15.5	1	855
CRAMP Kayaks 2 (Olowalu)	45.5	1	4.5	1420
CRAMP South Shallow 1 (Olowalu)	39	7	1.5	840
CRAMP South Shallow 2 (Olowalu)	10	2.5	0	815
Kapalua North	166	32.5	2.5	TMTC
Kapalua South	265	11.5	1.5	TMTC
Napili North	97.5	7.5	2.5	TMTC
Napili South	0	25	2	TMTC
Honokeana	9.5	11	1.5	TMTC
Runway	151	8.5	1.5	1620
Healthy North	35	22	1	1750
Bone Yard	115	28.5	13.5	1785
Healthy South	34.5	16.5	26	1495
Sand Channel	26	64.5	29	1425

Red highlights indicates levels exceeding the single sample limits.

Pink highlights indicates threshold reach for initiating 5 day geometric mean sampling.

Green highlights are plates with a 100 fold dilution (e.g., 1 ml filtered) was too concentrated with *Staphylococcus* to count or a confluent bacterial lawn on the filter plates.