

**Water Resources Research Center
Annual Technical Report
FY 2012**

Introduction

The Hawaiian Islands share the same water-related problems facing the population centers in the mainland U.S. and the world. Water shortages and quality problems are even more critical in Hawai'i and the Pacific because of their geographic isolation and small land areas. Therefore, the Hawaiian Islands can serve as a microcosm of what may already be a reality for a third of the population in the western and/or southwestern U.S.

Scientists at the University of Hawaii' Water Resources Research Center (WRRC) mainly address research related to water quantity and quality, including potential climate effects, and resource management. In addition to efforts of WRRC's staff, the Center integrates the expertise of the University's faculty to study problems related to sustainability of Hawaii's offshore recreational waters and onshore potable-water resources. Both current and future problems, affected mostly by climate change, are studied. Among these studies, a project is assessing the long-term aspects of high-elevation rainfall and climate change on the island of O'ahu. This work will bridge important gaps in hydroclimate and climate change knowledge for Hawai'i and the Pacific. Water quality studies have dominated our current research. Studies include developing an advanced surface tensiometer for measuring water quality; characterizing the physical, chemical, and biological properties of potable and recreational waters; addressing sewage contamination of Nawiliwili Stream and Kalapaki Beach, Kauai; evaluating a rapid qPCR method for enterococci for molecular markers for sewage contamination; assessing fate and transport of pharmaceutically active compounds in simulated bank filtration system; studying molecular, fluorometric and confocal microscopy analysis of microbial community composition and structure; using of UV disinfection for rain water catchment and stream water; and analyzing bioaccumulation and biotransformation of arsenic by marine algae in Hawaii. These studies will collectively advance the science in detection of contaminants and the assessment of their fate and transport. The practical benefits of some projects are obvious, including the use of UV disinfection, which addresses the need for potable water in rural areas.

Projects dealing with resource management include two studies assessing aquifer properties of the Northern Guam Lens Aquifer System and the Pearl Harbor Aquifer, Oahu. Such information is necessary for managing valuable water resources in systems with high competitive demands. This management class also includes a study dealing with the integrated management of groundwater and watershed management to meet the growing water demand. Others include a study aimed at reshaping the regulatory framework for Hawaii aquaculture ultimately aiming at enhancing greater community self-reliance in aquaculture production while sustaining environmental health. The last study in this group utilizes sedimentation data to promote reservoir sustainability and advance watershed science, as important steps towards managing surface water resources.

To support the technology transfer mission of WWRC, an Island Director's workshop/conference was held in 2011, which was aimed at strengthening communication between Pacific Islands' researchers and helping in identifying critical issues of major concerns. Another project is dealing with promoting water sustainability literacy by working towards the active participation of the community in water conservation efforts.

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The Hawaii NIWR 104-B program for FY 2012 funded nine new research projects, supported a multi-center conference on island water issues, and provided small amounts for technology transfer and administration. The grants provided through the USGS Water Resources Research Institute Program sets the foundation upon which other activities of the UHM WRRC are structured.

The funded research and no-cost extension projects are listed below.

1. Hydraulic Properties of the Northern Guam Lens Aquifer System, Territory of Guam, USA

The Northern Guam Lens aquifer system is the most important aquifer on the Island of Guam and currently supplies about 40 mgd of fresh groundwater mainly for public-supply. The resident population is expected to increase dramatically as the result of a proposed military buildup with as many as 70,000 residents added to the current estimate of about 171,000 residents. The groundwater demand is expected to increase proportionally with the additional population. This has led to concern over the long-term availability of water from the northern Guam aquifer. Hydraulic parameters such as hydraulic conductivity and storage parameters are essential elements of models used to manage groundwater availability and quality. Uncertainty in these parameters can result in erroneous model estimates and potential mismanagement of drinking-water resources. The objective of this work is to estimate aquifer properties of the northern Guam aquifer. A three-dimensional ground-water flow and transport model will be developed in a subsequent study to evaluate the availability of Guam's groundwater resources under several recharge and withdrawal scenarios. This study will identify hydrologic parameters to constrain numbers that can be used as input for this model.

Analyses of current and historical tidal-signal data in an array of wells widely distributed across the Northern Guam Lens Aquifer indicate that a lower-permeability limestone rim causes a significant tidal-damping effect at the boundary. For assigned specific yields of 0.01 to 0.4, hydraulic conductivity ranges from ~20 to 800 m/d for the former, and ~2,000 to 90,000 m/d for the latter. An argillaceous limestone unit exhibits intermediate conductivity. Assuming a specific yield of 0.35 for the Mariana Limestone, 0.05 for the Barrigada Limestone, and 0.005 for the Argillaceous Limestone and using the average aquifer thicknesses, the respective unique hydraulic-conductivity estimates would be 730, 12,000, and 170 m/d for the Mariana Limestone, Barrigada Limestone, and the Argillaceous Limestone. Thus, wells on the periphery consistently exhibit two orders of magnitude lower hydraulic conductivities than wells in the interior.

The hydraulic-conductivity contrast between the peripheral rocks and the interior rocks may be explained by (1) diagenetic reduction of hydraulic conductivity in the peripheral rocks, in contrast to (2) dissolutional enhancement of conductivity in the interior rocks, including (3) increasingly closer and more direct connection of porosity in the interior rocks to regional-scale porosity features, in contrast with (4) progressive organization and focusing of flow from the interior through the periphery through discrete secondary channels.

Hydraulic properties from the analytical Jacob solution are consistent with numerical model estimates. Results can thus be used to enhance the reliability of regional numerical density-dependent solute-transport and groundwater-flow models that investigate the effect of additional withdrawal in the Northern Guam Lens Aquifer under various recharge and withdrawal conditions in a subsequent study.

2. Long-term aspects of high-elevation rainfall and climate change, O'ahu

The climate and precipitation regime of the North Pacific and Hawai'i varies substantially across timeframes from annual to decadal and longer, and includes ENSO- and PDO-scale dynamics. Water resource planning

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over the long term (several decades) requires an understanding of the patterns and drivers of climate variation and change. Mountain rain is the crucial component of groundwater recharge on O'ahu. This research is aimed at better understanding long-term patterns of rainfall by reconstructing from peat swamp sediments (organic peat cores) the long-term eco-hydrological changes that have occurred at mountain sites on O'ahu. The study has been focusing on two sites, namely, Ka'au Crater in the southern Ko'olau Mountains and Ka'ala at the highest point of the Waianae Mountains. Of particular significance during this reporting period is the completed pollen work for the Ka'au Crater core and the recovery and preliminary analysis of the first-ever core from Ka'ala mountain. Of interest are changes in vegetation between 5000 and 6000 years ago, particularly in species in the palm family (Arecaceae) such as *Pritchardia* that suggest dramatic changes from very dry to very wet during this period. Changes in fossil pollen during this mid-Holocene period are contemporaneous with other geochemical data from this study as well as other climate proxies in Hawai'i, e.g. general cessation of reef accretion at multiple high-energy windward locations across the islands.

At the completion of the study, this work will bridge important gaps in hydroclimate and climate change knowledge for Hawai'i and the Pacific. This project has served as a platform for additional NSF funds.

3. Development of an advanced surface tensiometer for measuring water quality

Characterizing the physical, chemical and biological properties of potable and recreational waters plays a vital role in assessing and controlling water quality. The objective of this proposal is to develop an advanced surface tensiometer for assessing water quality. In addition to surface tension measurement, this surface tensiometer will be integrated with advanced microscopy and spectroscopy techniques for detection of specific contaminants. The resultant surface tensiometer will evaluate water quality based on four measurable parameters: 1) dynamic surface tension; 2) surface dilatational elasticity; 3) microscopy structure of adsorbed contaminant films using atomic force microscopy; and 4) chemical analysis of contaminants using Raman spectroscopy.

During the reporting period, the PI successfully developed the prototype of the CDS. The prototype consists of three primary modules: the optical module, the liquid handling module, and the environmental control module. The optical module, which consists of a high-resolution CMOS camera and a high-performance LED backlight, was developed with a separate grant. The liquid handling and the environmental control modules were developed in this project.

This advanced surface tensiometer allows determination of specific water contaminants and evaluation of water quality. Funds for this research were leveraged for competitive grants. PI Zuo received an NSF CAREER award on this topic.

4. Reshaping the regulatory framework for Hawaii aquaculture - water quality standards, coastal fishponds, and shellfish grounds

The goal of this project is to establish a broad-based, collaborative effort to promulgate new water quality regulations that will provide for greater community self-reliance in aquaculture production while sustaining environmental health. The four main objectives that we will achieve in pursuing this goal are (1) identify the different types of water quality standards revisions that could be proposed, including a survey of the practices in other jurisdictions; (2) document procedural roadmaps and scientific information needs for each type of revision identified; (3) analyze the potential for success in revising water quality standards for one or more coastal fishponds; and (4) estimate the resources needed to complete revisions on a wider scale.

Progress to date includes identifying potential water quality standard revisions regarding water-body types, protected uses, and biological monitoring and assessment methodologies. Potential revisions to the water quality standards that would support the advancement of Hawai'i aquaculture include: (1) changing the

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framework of water-body and marine bottom types and classes; (2) establishing and assigning designated uses (uses to be protected) on a more site-specific basis; (3) developing evaluative criteria that are explicitly tied to specific types, classes, uses, and sites; and (4) developing and implementing biological monitoring and assessment methods that are directly linked with use attainment decisions.

Mechanisms for initiating such regulatory change are available for agencies, elected officials, and citizens through a variety of legislative and administrative processes, but will usually require agency cooperation to be successful.

5. Addressing sewage contamination of Nawiliwili Stream and Kalapaki Beach, Kauai

This project is aimed at studying issues related to the pollution of Kalapaki Beach by discharges of cesspool wastes into the Nawiliwili watershed. The specific objectives of this project were (1) training of the PI, a new WRRC microbiologist, in advancing water quality testing methods, (2) collaboration of the PI with WRRC staff to gain experience regarding the Center's operations and to prepare proposals for research projects, and (3) collaboration with State agencies and plan for proposal submittal.

All freshwater sites, this includes all sampling sites except Kalapaki Beach (which was a marine site), were characterized by high concentrations of conventional indicator bacteria (*E. coli* and enterococci) throughout the study period. Concentrations of *E. coli* varied from less than 4 to 7920 CFU per 100 ml with a geometric mean varying from 196 to 1260 CFU per 100 ml between sites and concentrations of enterococci varied from 41 to 6040 CFU per 100 ml with a geometric mean varying from 76 to 1928 CFU per 100 ml between sites. Hence, concentrations of indicator bacteria were elevated, except at the marine site (S-5 Kalapaki Beach) where concentrations of enterococci were relatively low (4–26 CFU per 100 ml, with a geometric mean of 9 CFU per 100 ml) (Table 1). These results are comparable to an earlier study, although no elevated fecal indicator bacteria concentrations were detected at the marine site. Current Hawaii recreational freshwater water quality standards are based on enterococci and explicitly states that no sample should exceed 89 CFU of enterococci per 100 ml and geometric mean of samples collected over 25–30 days should not exceed 33 CFU of enterococci per 100 ml. All samples collected, including the control sample from the pristine environment, exceeded the standard based on the geometric mean throughout the study period. The standard based on the single sample maxima was also exceeded in all freshwater samples, except for one sample collected from the Puali Stream and three out of four samples collected at the pristine control site (Lawai Stream). These findings collectively indicate that current water quality standards are not suitable for Hawaii due to high environmental background of indicator bacteria, and therefore have little relevance to the actual health risk. Current Hawaii recreational marine water quality standards are based on enterococci and explicitly states that no sample should exceed 104 CFU of enterococci per 100 ml and geometric mean of samples collected over 25–30 days should not exceed 35 CFU of enterococci per 100 ml. Samples collected at the Kalapaki Beach did not exceed the standards during the study.

6. Island Director's workshop/conference

The conference "Water Resource Sustainability Issues on Tropical Islands" was held in Honolulu, Hawaii, November 14–16, 2011. The conference was held in collaboration with water centers in Guam, Puerto Rico, and U.S. Virgin Islands. The issue of sustainability is especially critical for islands due to resource limitation and water vulnerability to contamination. Further, alternative energy sources, such as bio-energy, have added more strain on water resources. Demands are multiplying due to population growth and urbanization. The issues related to the coordinated management of surface water and groundwater are of prime importance. Water resources are particularly sensitive to climate change due to islands' particular nature. Water scarcity and vulnerability to drought, flooding, and other natural disasters considerably increase as island size decreases. Major factors affecting water resources include physical island characteristics, such as size and topography, climate, and human impact. Climate change can lead to further degradation of water quality,

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which is already a major problem in many islands. Contamination originates from point and non-point sources. Pollution sources include discharges of untreated or partially treated wastewater and animals farms, inadequate solid waste disposal sites, agricultural chemicals, leakage of petroleum products and toxic chemicals, sediment erosion, and saltwater intrusion. The small size and steep slopes of catchments on high islands enable water and pollutants to move quickly to downstream areas. The highly permeable soils and shallow water tables on small coral islands facilitate the rapid migration of pollutants to the subsurface. The reversal of these negative impacts is difficult and time consuming. Pollution affects human health due to microbiological contamination and elevated chemical levels in water supplies. High turbidity and suspended solids are experienced by consumers after periods of heavy rainfall. The effectiveness of water supply intakes and treatment systems is compromised by high-suspended sediment loads, leading to higher costs of providing clean, safe water supplies. Sedimentation in water supply reservoirs and rivers lead to disturbances in upstream catchments. Finally, sediments, bacteria, and chemicals are negatively affecting riverine and coastal environments.

Proceedings are being prepared based on presentations made at the meeting.

7. Evaluation of rapid qPCR method for enterococci with correlative assessment for molecular markers for sewage contamination in selected environmental water samples from Hawaii

This project will provide an assessment of the rapid qPCR test for enterococci in Hawaii and therefore prepare the local government agencies to the changes in the federal water quality criteria. The PI will work with these agencies to provide training as well as relevant information to their public outreach programs as requested. Results of the study will enable State Department of Health to assess suitability of the USEPA's novel rapid test for enterococci for Hawaii and if the test should be incorporated into the State's water quality regulations. Novel molecular tests are expected to improve current microbial water quality monitoring programs and are needed for meaningful water management decisions in Hawaii. While the technology addressed in this project is applicable in all high priority areas identified by the Water Resources Research Institute Program, the project is centered on 'Water Quality' and has the following objectives: 1. Establish and evaluate performance of rapid qPCR test for enterococci (USEPA draft protocol A) in parallel with cultivation-based assays in Hawaii. 2. Establish and evaluate qPCR based MST tools in Hawaii.

This project is ongoing. Progress to date includes sample collection, protocol setup for data analysis, and steps for validation of source tracking markers.

8. Promoting water sustainability literacy

The purpose of this project is to articulate and disseminate both the challenges to and importance of sustainability of freshwater supplies at a Water Forum on the campus of the University of Hawaii at Manoa (UHM) Sustainability Courtyard (SC). The primary goal is the promotion of water resource management literacy. The interdisciplinary project involved collaboration among UHM faculty and students, as well as Federal and State agencies involved in water management. The SC as a center of campus engagement is an educational benefit to facilitate understanding of key issues associated with water management in the State of Hawai'i. The study included water-sustainability literacy events, which were well attended by a total over two hundred people, averaging about 20 people per event. Among the principal findings is a need to improve coordination among members of the campus community who are already engaged with the topics of environmental education, sustainability literacy, and water resource management. Of particular significance is the timeliness of the topics and events in relation to the current development of on-campus and off-campus academic programs, course proposals, and research and service projects. The water literacy events contributed to the achievement of sustainability education and awareness training that is promoted in the current campus strategic plan. The topics covered are linked with specific objectives established in the UHM Chancellor's "Sustainability Policy Statement" such as maximizing water conservation, water efficiency, and best

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management practices for storm-water storage, recharge, and reuse. In Fall 2012, the water sustainability literacy project culminated in a Sustainability Open House that showcased seven off-campus student internships and fifteen on-campus projects initiated by students (December 5-6, 2012). The student prepared and presented of web-based resources, including YouTube videos, PowerPoint slides, conference posters, and project base maps that can be used for future reference by the campus community.

9. Satisfying growing water demand through integrated groundwater and watershed management

Sustaining water availability at current prices in the face of growing demand and declining resources is highly unlikely. Therefore, this research aims to develop a management framework with the objective of conserving water resources in a manner that maximizes water users' benefits over time, given projected effects of a continuously changing climate. Specifically, the research considers long-term planning for efficient extraction of groundwater and timing for the development of groundwater alternatives, such as desalinated water, when recharge is declining. Recharge-enhancing watershed conservation would further help to moderate scarcity, resulting from the dual effect of increasing demand and declining recharge, and thus positively affect social welfare. By comparing welfare, estimates under different assumptions about recharge, including different rates of decline and maintenance, it is possible to indirectly estimate a lower bound for ecosystem benefits associated with hypothetical conservation projects.

The study considered a number of scenarios and completed sensitivity analyses. Factors evaluated include year of implementing desalination, the net present value of the resource, and the benefit of maintaining recharge at certain level. For the baseline case, even when groundwater is abundant and recharge is projected to decline moderately, the value of watershed conservation can be substantial. Sensitivity analyses concluded that when recharge decline is projected to be relatively small and groundwater is abundant, changes to some parameters do not substantially affect the optimal management strategy. Furthermore, the value of maintaining recharge is higher if recharge is expected to decline by a larger amount if watershed management is excluded. The study concluded with recommendations to generalize the analysis to include the whole island of O'ahu including the characterization of natural flow between the various groundwater sectors, distribution or allocation of water between sectors, and the sequencing of all available water sources over space and time.

10. Fate and transport of pharmaceutically active compounds in simulated bank filtration system

Riverbank filtration (RBF) represents a natural filtration technique that has been used for providing drinking water for more than a century in Europe and half a century in the United States. RBF uses the riverbed and the underlying aquifer as natural filters to remove pollutants present in the surface water. Straining, colloidal filtration, sorption and microbial degradation contribute to the removal and attenuation of these contaminants. This study used a two side-by-side slab to study the fate and transport of different pharmaceutically active chemicals under different environmental conditions occurring at the RBF site. The findings can be extrapolated to seawater filtration typically used as pre-treatment for membrane filtration.

Among other results, the study showed that the environmental behavior of phenazone and caffeine was highly impacted by redox conditions and seasonality at the bank filtration site. Under anaerobic conditions, both chemicals were persistent and low/limited removal (less than 15%) was observed. While under aerobic conditions, both chemicals were completely removed. Caffeine and phenazone were completely removed within 20 days during the summer, while only phenazone was removed during the winter. Phenazone required an acclimation period of 15 and 65 days during summer and winter, respectively. In the presence of cold temperature, the removal rate of phenazone was also slower compared to the removal rate observed during the summer.

11. Molecular, fluorometric and confocal microscopy analysis of microbial community composition and structure

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While periodic assessment of the effect of the effluent from the Sand Island sewage outfall on the benthic invertebrate population has been performed, there has not been an assessment of the possible shift in the marine microbial community structure. In the last published assessment of the microbial activity at the outfall, the investigators concluded “Although the activity of the microbial populations does not seem to have been affected drastically by the input of sewage effluent, we have no indication of its effect on the microbial community structure. The fate of the large microbial community on the effluent itself is unknown.” This is a serious shortcoming, as a shift in the microbial community structure can be pathological and has serious ecological implications. Since 1985, methods have been developed to analyze the microbial community structure at the outfall and control sites. The relationships between marine microbes and human health will increasingly be important, as human populations rise and NSF has made Oceans and Human Health a priority. The objective of this proposal is to assess the microbial community by analyzing the biological films that result for colonization and settlement of water column microbes and propagules.

To analyze changes in marine microbial communities, bacteria at impacted urban reefs were compared to a pristine site with DGGE analysis. The results indicated that a total of 57 different 16S rDNA patterns were detected from the study sites. The compositions of microbial communities from Hawaii Kai reef were clearly distinguished from the other study sites within 12.8% of similarity. On the other hand, the compositions of microbial communities between Waikiki and Ala Wai Canal showed 37.7% similarity. Therefore, the results strongly indicate the compositions of microbial communities are distinct among the marine microbial community at these different study sites, and at relatively fine scales.

For DNA sequence analysis based on the excised DGGE bands, a total of 8 distinct DGGE fragments were excised and identified from the study sites. A diverse group of bacterial communities was also found in the study sites during the experimental period (*Synechococcus* sp. CC9311, *Shewanella denitrificans* OS217, *Saprospira grandis*, *Marivirga tractuosa* DSM 4126, *Flavobacterium johnsoniae* UW101, *Vibrio vulnificus* CMCP6, *Synechococcus* sp. PCC7002, and *Pseudomonas mendocina* ymp). *S. denitrificans* OS217, *V. vulnificus* CMCP6, and *P. mendocina* ymp indicate that the microbial community structure from the study sites can be pathological and could have serious ecological implications.

The biofilm microscopy for community structure results indicated complex chemistry of the EPS from diatoms and bacterial cells/EPS. The specificity of the lectin binding allows us to conclude that the composition of the biofilm matrix contains glycoconjugates with mannose/glucose moieties that is also localized at bacterial cells and EPS. The glycoconjugates with sialic acid residues are most concentrated in the diatom and bacterial EPS. Results of water chemistry and PAM indicated that Ala Wai Canal showed high levels of inorganic nutrients and the highest algal photosynthesis level (ETR_{max}) among the study sites. Therefore, we could conclude Ala Wai Canal is eutrophic via inputs of impaired water or with sewage spills, and the populations of autotrophic organisms are higher than at other study sites.

On Oahu, including sites that have potentially elevated wastewater inputs, indications of pathological and serious ecological implications were found (e.g., *Vibrio vulnificus* and *Pseudomonas mendocina*) in the microbial community structure, especially at Ala Wai Canal and at Waikiki. However, how quickly microbial communities shift from impacted invasive alien algae to healthy states, remains unknown.

12. Acquire sedimentation data to promote reservoir sustainability and advance watershed science

The objective of this study is to compile sedimentation data from various sources to assess reservoir sustainability on the Hawaiian Islands as an important step towards facilitating watershed studies. The approach includes the following.

1) obtain design and as-built bed elevations for all Hawaii reservoirs that are listed in the National Inventory of Dams (NID); 2) obtain other reservoir design factors (drainage area sediment yield, source sediment and

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bed sediment bulk density, and reservoir storage capacity loss rate) for the same facilities; 3) obtain corresponding data from subsequent bathymetric surveys, watershed analyses, sediment sampling, and hydrologic calculations; 4) organize the collected information into a database that mimics the structure of National Reservoir Sedimentation Database RESSED; 5) perform a gap analysis of the database records to identify remaining data needs; 6) develop a sampling and analysis plan for a field and archival investigation to fill gaps in the historic record and establish new baselines for reservoir physical characteristics; and 7) collaborate with other investigators, agencies, and interested parties to prepare and submit follow-on proposals for implementing the sampling and analysis plan and loading completed records to RESSED.

Data compiled from the state inventory contains estimates of original reservoir storage capacity that provide a useful basis for analyzing the distribution of storage capacity among islands, owners, purposes, irrigation service areas, receiving waters, watershed contributing areas, and other significant institutional and physical characteristics.

There is a great uncertainty about bed elevation, which is not routinely included in the data sources. In some cases, bed elevation may be roughly inferred from site topography and existing inventory data about dam height, dam base elevation, and reservoir depth. In addition, initial database queries, literature searches, and personal contacts indicate that it would take considerable effort (beyond the resources of the current project) to acquire reasonably complete information about each of the 140 reservoirs in the state inventory. Therefore, the PI is first classifying the universe of reservoirs according to levels of information availability, then targeting a subset of reservoirs in each class for focused investigation, in order to estimate the degree of effort that might be required to complete data collection for all reservoirs.

13. Usage of UV disinfection coupled with rain water catchment and stream water in rural areas

Water is an essential component for living organisms. From the beginning of civilization, humans have settled close to water sources. Unfortunately, in many developing nations, available water is unfit for human consumption because of high bacterial content. Lack of good quality water can also be a problem in the more remote areas of developed countries. An economical ultraviolet (UV) disinfection system run by solar power may provide a means of disinfecting the available water and making it suitable for drinking. With proper dosage, UV irradiation is an effective bactericide and virucide and does not contribute to the formation of toxic by products. The UV unit can be used as a point of use device for a single household or as a final treatment on a slow sand filter that would be large enough to serve a small community.

Based on this research, the homemade UV unit was cheap and easy to make. It did not require any sophisticated equipment. The unit performance was comparable to the commercial UV unit. However, the solar panel was not able to provide consistent and adequate power to the unit. Instead of looking for a bigger or better performing solar panel, it was recommended to look for a germicidal lamp with a lower wattage. A submersible germicidal lamp that can be immersed into the sample is ideal.

14. Bioaccumulation and biotransformation of arsenic by marine algae in Hawaii

Marine algae are known to bio-accumulate and transform Arsenic (As). AS was used in earlier decades as a pesticide on agricultural fields in Hawai'i. Macroalgae collected at some sites on the shore of O'ahu have been found to contain elevated concentrations of As. Runoff of contaminated former agricultural fields is the suspected source. Furthermore, several species of macroalgae are commonly gathered and consumed as human food. The approach adopted included collecting samples of algae species from beaches around the island of O'ahu. Samples were analyzed for the amount and species of arsenic compounds they contained. Findings of these analyses were analyzed about the geographic distribution of the samples and historical agricultural activities in the areas surrounding the collection sites.

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The concentration of total arsenic found in the single sample analyzed to date was 10 g/g. This is in general agreement with concentrations reported by an earlier analysis done for The Nature Conservancy in Hawaii, and is relatively low. Other researchers have reported significantly higher concentrations in commercially available edible dried seaweed (e.g. García-Salgado et al.). An edible kelp was analyzed for the sake of comparison and this was found to have a total arsenic concentration of 65 g/g. The analysis was conducted on a non-edible species from Hawaii (*Gracilaria salicornia*). We intend to follow up with analyses of edible species during the next year.

Perhaps the most significant contribution of the project was the development of analytical techniques by the project chemist, John W. Scott, at the Illinois Sustainable Technology Center, which made possible improved speciation of total arsenic into its various forms. A preliminary attempt using trifluoroacetic acid and methanol to extract the arsenic from the algae sample followed by liquid chromatography inductively coupled plasma mass spectrometry (LC-ICPMS) yielded unsatisfactory results (the extraction method caused changes in the forms of arsenic present). Furthermore speciation of the extracts from these experiments by LC-ICPMS indicated that the forms of arsenic present were not amendable to the methods used. Subsequently the extraction protocol was modified and the total arsenic analysis was conducted using inductively coupled plasma mass spectrometry ICP-MS. Arsenic speciation was achieved with a liquid chromatography system interfaced to the ICP-MS instrument operated in a transient acquisition mode. The various species of arsenic detected using this system accounted for 70% of the arsenic in the algae. In addition to arsenite, monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA), we observed two unknown forms of arsenic in both the algae from Hawaii and the edible kelp, however identification of these was not possible using the above methods.

15. Determination of groundwater fluxes and evaluation of the effectiveness of low-permeability valley-fills in the Pearl Harbor Aquifer area, Oahu

This study is aimed at assessing the effects of stream valleys that are filled with alluvium below the water table that act as hydrologic barriers to cross-valley groundwater flow. Weathered basalt underneath the streambed contributes to the permeability contrast under the valley fill with respect to the otherwise high-permeability basalt aquifer. The scope of work includes (1) developing a regional numerical groundwater model that quantifies groundwater fluxes to the Pearl Harbor Aquifer from adjacent areas, and (2) analyzing groundwater-level data to evaluate the hydrologic effectiveness of valley-fill barriers, including those associated with Waimano, Waimalu, and Kalauao Streams.

The study, which is in progress, includes developing a three-dimensional island-wide MODFLOW model with the focus on groundwater areas adjacent to the Pearl Harbor Aquifer. The numerical model is capable of simulating groundwater flow and the freshwater-saltwater interface using the Saltwater-Intrusion (SWI) package. The steady-state model of the recent hydrologic conditions is calibrated using observed groundwater levels, vertical salinity profiles, and estimated base flows of streams. Upon successful calibration, groundwater fluxes into the Pearl Harbor Aquifer can be determined for recent conditions. The effects of predevelopment conditions on the location of the groundwater divide between leeward and Pearl Harbor side in the Koolau high-level water area can be tested.

Recent synoptic water-level surveys in the Pearl Harbor Aquifer by the USGS and water levels measured on opposite sides of valley-fills will be used to characterize the effectiveness of the alluvium as a hydrologic barrier. Moreover, continuously measured water levels will be analyzed to evaluate the cross-boundary effects of groundwater withdrawals. After removing environmental stresses that influence water levels other than groundwater withdrawals (e.g., barometric pressure, recharge events), the water-level time series can be investigated for signs of drawdown and recovery across valley fills.

Hydraulic Properties of the Northern Guam Lens Aquifer System, Territory of Guam, USA

Basic Information

Title:	Hydraulic Properties of the Northern Guam Lens Aquifer System, Territory of Guam, USA
Project Number:	2010HI316S
Start Date:	6/30/2010
End Date:	12/31/2012
Funding Source:	Supplemental
Congressional District:	Hawaii 1st
Research Category:	Ground-water Flow and Transport
Focus Category:	Groundwater, Hydrology, Water Supply
Descriptors:	Aquifer Parameter Estimation, Tidal Responses, Aquifer Test
Principal Investigators:	Aly I El-Kadi

Publications

1. Rotzoll, K., J. Jenson, and A.I. El-Kadi, 2011, Estimating hydraulic properties of the Northern Guam Aquifer by analysis of ocean-driven groundwater-level fluctuations, Abstract H34E 08, presented at 2011 Fall Meeting, AGU, San Francisco, CA, 5-9 Dec.
2. Rotzoll, K., S.B. Gingerich, J. Jenson, and A.I. El-Kadi, Estimating hydraulic properties from tidal attenuation in the Northern Guam Lens Aquifer, Territory of Guam, USA, (in preparation for submission to Hydrogeology Journal).
3. Rotzoll, K., J.W. Jenson, and A.I. El-Kadi, 2011, "Estimating hydraulic properties of the northern Guam aquifer by analysis of ocean-driven groundwater-level fluctuations", Abstract H34E-08, presented at 2011 Fall Meeting, AGU, San Francisco, CA, Dec. 5-9.
4. Rotzoll, K., S.B. Gingerich, J.W. Jenson, and A.I. El-Kadi, 2013, "Estimating hydraulic properties from tidal attenuation in the northern Guam lens aquifer, Territory of Guam, USA", Hydrogeology Journal, DOI: 10.1007/s10040-012-0949-9.
5. Jenson, J.W., D. Taboro'i, K. Rotzoll, J.E. Mylroie, and S.B. Gingerich, 2013, "A hypothesis for carbonate island karst aquifer evolution from analysis of field observations in northern Guam, Mariana Islands", International Symposium on Hierarchical Flow Systems in Karst Regions, Budapest, Hungary, Sept. 2-7.

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The Northern Guam Lens aquifer system is the most important aquifer on the Island of Guam and currently supplies about 40 mgd of fresh groundwater mainly for public-supply. The resident population on the island of Guam has been increasing, and is expected to increase dramatically as the result of a proposed military buildup with as many as 70,000 residents added to the current estimate of about 171,000 residents. The groundwater demand is expected to increase proportionally with the additional population. This has led to concern over the long-term availability of water from the northern Guam aquifer (Gingerich and Jenson 2010).

Problem and Research Objectives

Hydraulic parameters such as hydraulic conductivity and storage parameters are essential elements of models used to manage groundwater availability and quality. Uncertainty in these parameters can result in erroneous model estimates and potential mismanagement of drinking-water resources. The objective of this work is to estimate aquifer properties of the northern Guam aquifer.

A three-dimensional ground-water flow and transport model will be developed in a subsequent study to evaluate the availability of Guam's groundwater resources under several recharge and withdrawal scenarios. This study will identify hydrologic parameters to constrain numbers that can be used as input for this model.

Methodology

Tidal-signal attenuation is conveniently used to estimate hydraulic properties, such as hydraulic conductivity and storage parameters of coastal aquifers and to determine the distance of tidal influence into the aquifer (e.g., Rotzoll et al. 2008). Jacob (1950) provided a now classic analytical solution for water levels in a one-dimensional, homogeneous, isotropic, confined, and semi-infinite aquifer with a sharp boundary subject to oscillating forcing. Although techniques to analytically calculate tidally influenced groundwater levels have become more sophisticated by adding more details to the simplified solution, the relatively simple model remains useful for estimating aquifer hydraulic properties from measured groundwater-level fluctuations. Groundwater levels with notable tidal influence from 34 wells in the Northern Guam Lens Aquifer were analyzed.

Principal Findings and Significance

Analyses of current and historical tidal-signal data in an array of wells widely distributed across the Northern Guam Lens Aquifer indicate that a lower-permeability limestone rim causes a significant tidal-damping effect at the boundary. For assigned specific yields of 0.01 to 0.4, hydraulic conductivity ranges from ~20 to 800 m/d for the former, and ~2,000 to 90,000 m/d for the latter. An argillaceous limestone unit exhibits intermediate conductivity. Assuming a specific yield of 0.35 for the Mariana Limestone, 0.05 for the Barrigada Limestone, and 0.005 for the

Argillaceous Limestone and using the average aquifer thicknesses, the respective unique hydraulic-conductivity estimates would be 730, 12,000, and 170 m/d for the Mariana Limestone, Barrigada Limestone, and the Argillaceous Limestone. Thus, wells on the periphery consistently exhibit two orders of magnitude lower hydraulic conductivities than wells in the interior.

The hydraulic-conductivity contrast between the peripheral rocks and the interior rocks may be explained by (1) diagenetic reduction of hydraulic conductivity in the peripheral rocks, in contrast to (2) dissolutional enhancement of conductivity in the interior rocks, including (3) increasingly closer and more direct connection of porosity in the interior rocks to regional-scale porosity features, in contrast with (4) progressive organization and focusing of flow from the interior through the periphery through discrete secondary channels.

Hydraulic properties from the analytical Jacob solution are consistent with numerical model estimates. Results can thus be used to enhance the reliability of regional numerical density-dependent solute-transport and groundwater-flow models that investigate the effect of additional withdrawal in the Northern Guam Lens Aquifer under various recharge and withdrawal conditions in a subsequent study.

Publications Cited in Synopsis

Gingerich, S.B., and J.W. Jenson, 2010, Groundwater availability study for Guam; Goals, approach, products, and schedule of activities, U.S. Geological Survey Fact Sheet 2010–3084, 4 p.

Jacob, C.E., 1950, *Flow of ground water*, in *Engineering Hydraulics*, edited by H. Rouse, pp. 321–386, John Wiley, Hoboken, New Jersey.

Rotzoll, K., A.I. El-Kadi, and S.B. Gingerich, 2008, “Analysis of an unconfined aquifer subject to asynchronous dual-tide propagation,” *Ground Water*, 46(2), 239–250.

Long-term aspects of high-elevation rainfall and climate change, O'ahu

Basic Information

Title:	Long-term aspects of high-elevation rainfall and climate change, O'ahu
Project Number:	2011HI318B
Start Date:	3/1/2011
End Date:	2/28/2014
Funding Source:	104B
Congressional District:	Hawaii 1st
Research Category:	Climate and Hydrologic Processes
Focus Category:	Climatological Processes, Wetlands, Drought
Descriptors:	
Principal Investigators:	David Beilman

Publication

1. Schubert, Olivia, 2012, "Vegetation reconstruction of Ka'au Crater through the Holocene," MS Thesis, Department of Geography, College of Social Sciences, University of Hawaii at Manoa, Honolulu, HI.

Problem and Research Objectives

The climate of the Hawaiian Islands, particularly rainfall, is notoriously dynamic across geographic space (Giambelluca et al., 2011; Figure 1) and has long been recognized to be strongly influenced by changes in Pacific atmospheric circulation (Chu, 1995). Variation over time includes dynamics ranging from annual to decadal and longer, including ENSO and PDO scale dynamics (Chu and Chen, 2005). Mountain rain is the crucial input for groundwater recharge in Hawaii (Giambelluca et al., 1993) and the ultimate source of much-demanded water for the City and County of Honolulu. Water resource planning into the future (years and decades) requires a long-term understanding of the patterns and drivers of climate variation and change.

In this study, we seek to better understand long-term patterns of rainfall by reconstructing from peat swamp sediments (organic peat cores) the long-term ecohydrological changes that have occurred

at mountain sites on Oahu. We have been focusing on two study sites laid out in our original proposal; Kaau Crater in the southern Koolau Mountains and Kaala at the highest point of the Waianae Mountains (Figure 1). Of particular significance during this reporting period is the completed pollen work for the Kaau Crater core, the graduation of a Master's student supported by this grant, and the recovery and preliminary analysis of the first-ever core from Kaala mountain.

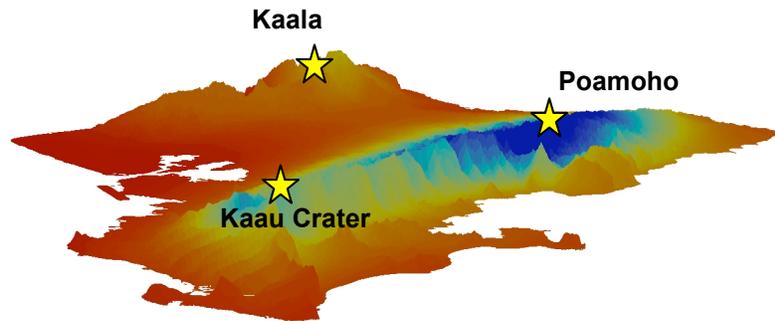


Figure 1. Three-dimensional rendering (base heights exaggerated) of Oahu, Hawaii viewed from the southeast showing our study sites. Coloring shows mean annual rainfall (red = drier, blue = wetter) from 1978 to 2007 (Giambelluca et al., 2012).

Methodology

We are employing two main lines of enquiry: 1) fossil pollen abundances reflecting changes in local vegetation over time and 2) stable isotope and organic geochemistry of bulk sediment and specific biomolecules (leaf waxes; *n*-alkanes). Fossil pollen work has concentrated on the last 8,000 years of well-dated sediments from Kaau Crater. Stable isotope geochemistry work has also focused on Kaau Crater sediments (see principle findings). Sediments have been collected from Poamoho Pond (1 m; not as promising as first expected) and from Kaala (1.5 m; very promising). We have conducted preliminary leaf wax organic chemistry on the Kaala core via a new collaboration with Dr. Jon Nichols at Columbia University (see principle findings).

The position of the water table is one of the critical links between rainfall/hydrology, plant growth and soil organic matter dynamics, and thus the accumulation of organic sediments and their geochemical character. In recognition of this importance, we added a secondary component to this study in 2012 to monitor the water table fluctuations (and other variables) with rainfall at

Kaau Crater, Poamoho, and Kaala. With support from other sources, automated loggers recording water table, air and soil temperature, relative humidity, and photosynthetically active radiation were installed at Kaau Crater in July 2011, Poamoho in September 2011, and Kaala in September 2012 (see principle findings).

Principle Findings of Significance

Fossil Pollen

Pollen of approximately 34 different families of plants was discovered in Kaau Crater sediments, spanning the last 8,000 years (Figure 2). Twenty peat samples were processed by standard acid-base-hydrochloric_acid-acetolysis preparation and 20 assemblages were counted to show vegetation response over time. Master's student Olivia Schubert conducted this work as part of her thesis; Ms. Schubert graduated in December 2012 (see Student Support). Of interest are changes in vegetation between 5000 and 6000 years ago, particularly in species in the palm family (Arecaceae) such as *Pritchardia* that suggest dramatic changes from very dry to very wet during this period. Changes in fossil pollen during this mid-Holocene period are contemporaneous with other geochemical data from this study (see geochemistry section below) as well as other climate proxies in Hawaii, e.g., general cessation of reef accretion at multiple high-energy windward locations across the islands (Rooney et al., 2004). A manuscript written by the supported student on this project (see Student Support Section) for submission to a peer-reviewed journal (target: Journal of Paleolimnology, impact factor: 1.898) resulting from this pollen work is expected in 2013.

Stable Isotope Geochemistry

Nitrogen stable isotope values ($\delta^{15}\text{N}$) of bulk sediment from Kaau Crater show surprising variation over time, including enriched values in the oldest organic sediments (that have been subject to microbial decay for thousands of years) and those sediments disturbed at the surface. Most interestingly, a highly enriched departure is evident around 5000 years ago. Such an enrichment in ^{15}N is consistent with a greater degree of microbial processing of organic matter (trophic level enrichment of microbial biomass) and is consistent with a drop in water table around 5000 years ago, a period suggested by Uchikawa et al. (2010) to have been dominated by dry vegetation on Oahu's leeward Ewa Plain, and during a period of highly variable PDO and El Niño conditions (Figure 3).

Our isotopic measurements of plant leaf waxes (*n*-alkanes) extracted from Kaau Crater sediments have yielded promising early results. *n*-alkane abundance has ranged from 234 $\mu\text{g g}^{-1}$ to below detectible limits for samples between 14 cm and 390 cm in the collected profile, with leaf wax composition (abundance of different chain lengths) and hydrogen isotope values surprisingly variable; ranging from -132 to -192‰. An overall depletion in mean $\delta^2\text{H}_{n\text{-alkane}}$ over the last 8000 years is consistent with an overall drying pattern over thousands of years, which is also suggested by Uchikawa et al. (2010) for leeward Oahu.

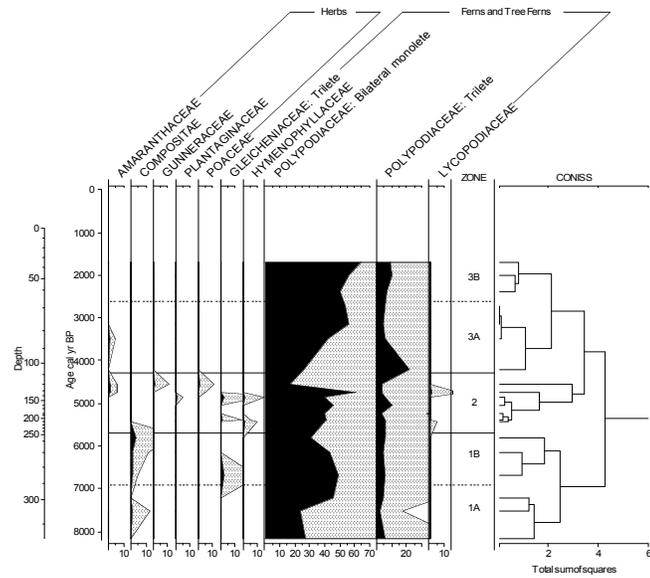
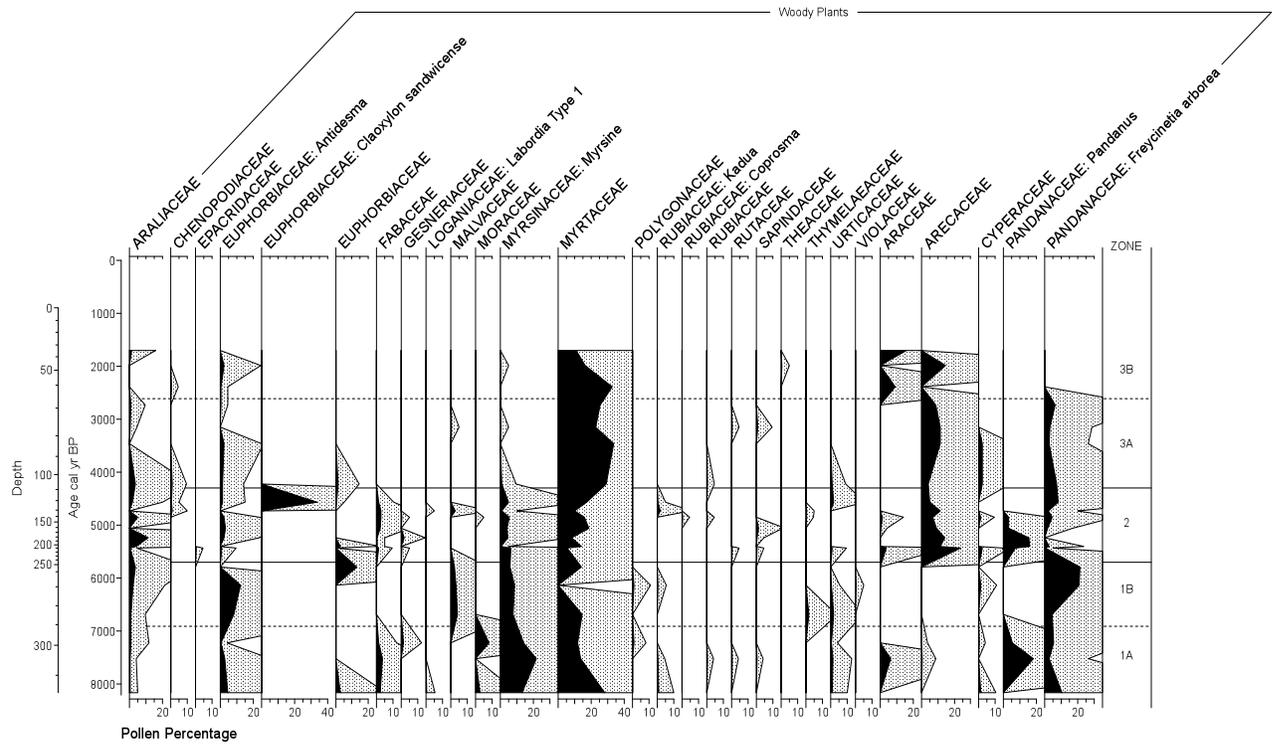


Figure 2. Fossil pollen abundance changes over time in a Holocene-aged core from Kaau Crater, Oahu, Hawaii. The chronological control of the profile is provided by 17 radiocarbon dates.

Organic Geochemistry

A potentially powerful paleoscience approach using terrestrial sediments is to extract and analyze leaf waxes at high resolution. We seek to test the hypothesis that the overall drying of Oahu climate has been punctuated by multi-decadal or longer periods of drought (Figure 3) and that these changes will be expressed in the distribution of leaf waxes and their stable isotope values. With support from this WRRIP grant, we have developed the capability to perform total lipid extractions and *n*-alkane separations. In April and May 2013 we extracted and separated *n*-alkanes from 40 samples. In May 2013 these were analyzed by GCMS for alkane abundances and distributions. Of significance in these samples was the large abundance of short-chain *n*-alkanes (average C-15 contribution to the total *n*-alkane distribution by mass was 47%) that suggest a unicellular/algae source of organic matter. Of additional significance was a difference in vascular plant (longer chain; C-25 to C-35) *n*-alkane distributions between sites. For example, Kaala mountain (drier location) shows much more variation in Average Chain Length or ACL (27.8 to 30.0) than the surprisingly consistent ACL of Kaau Crater sediments (wetter location; 28.7 to 29.7). This suggests that the vegetation at Kaala was more sensitive to change in the past. We plan to make GC-IRMS measurements of hydrogen isotope values of different chain lengths through our new collaboration with Dr. Nichols. The remaining funds in this small exploratory grant will be used for these analyses and will also provide ‘proof-of-concept’ data for future proposals (see future funding section).

Rainfall and Water Table Dynamics

At Kaau Crater, the water balance of the crater swamp consists of inputs (rainfall, surface water, and groundwater) and outputs (evapotranspiration and runoff) that determine water table dynamics and processes that affect sedimentation, plant growth and soil decomposition. Our monitoring of water table depth changes at Kaau Crater shows sensitive response to rainfall changes (Figure 4), but also observe that prolonged rainfall does not result in proportionate storage (excess is lost to runoff) and that prolonged periods with little rainfall show a rapid response of water table drop (see Figure 4; January and February 2012) rather than buffering from groundwater inputs or the water-holding capacity of the peatswamp. Water table dynamics (and the impact on ecohydrology and ecosystem processes) may be more sensitive to dry periods than wet periods. Continued monitoring over dry and wet seasons will help address the drivers of water table changes and impacts.

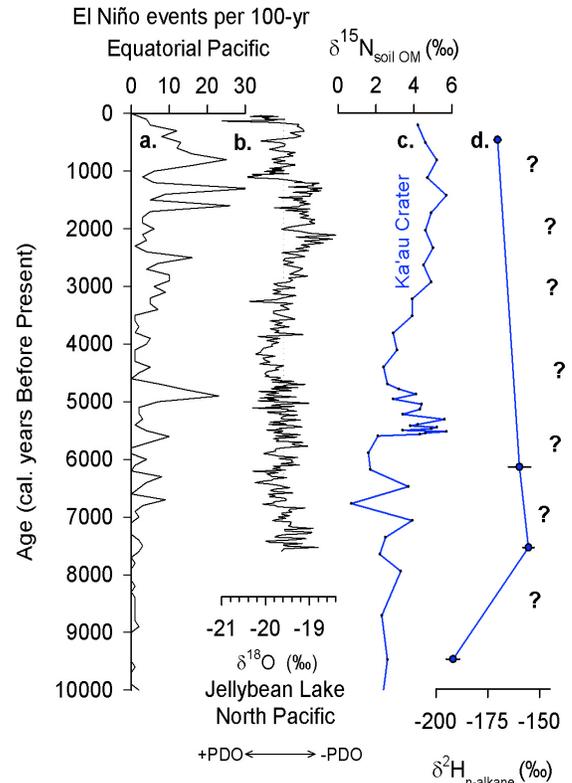


Figure 3. Long-term reconstructions of ENSO and PDO and isotope data from Kaau Crater. **a.** El Niño events (Moy et al., 2002). **b.** PDO reconstruction (Anderson et al., 2005). **c.** $\delta^{15}\text{N}$ values of Kaau Crater peatswamp sediment organic matter (OM). **d.** $\delta^2\text{H}$ values of leaf waxes from Kaau Crater, which we are currently filling in at higher resolution. Note the dry anomaly (greater $\delta^{15}\text{N}_{\text{OM}}$) around 5,000 yr BP.

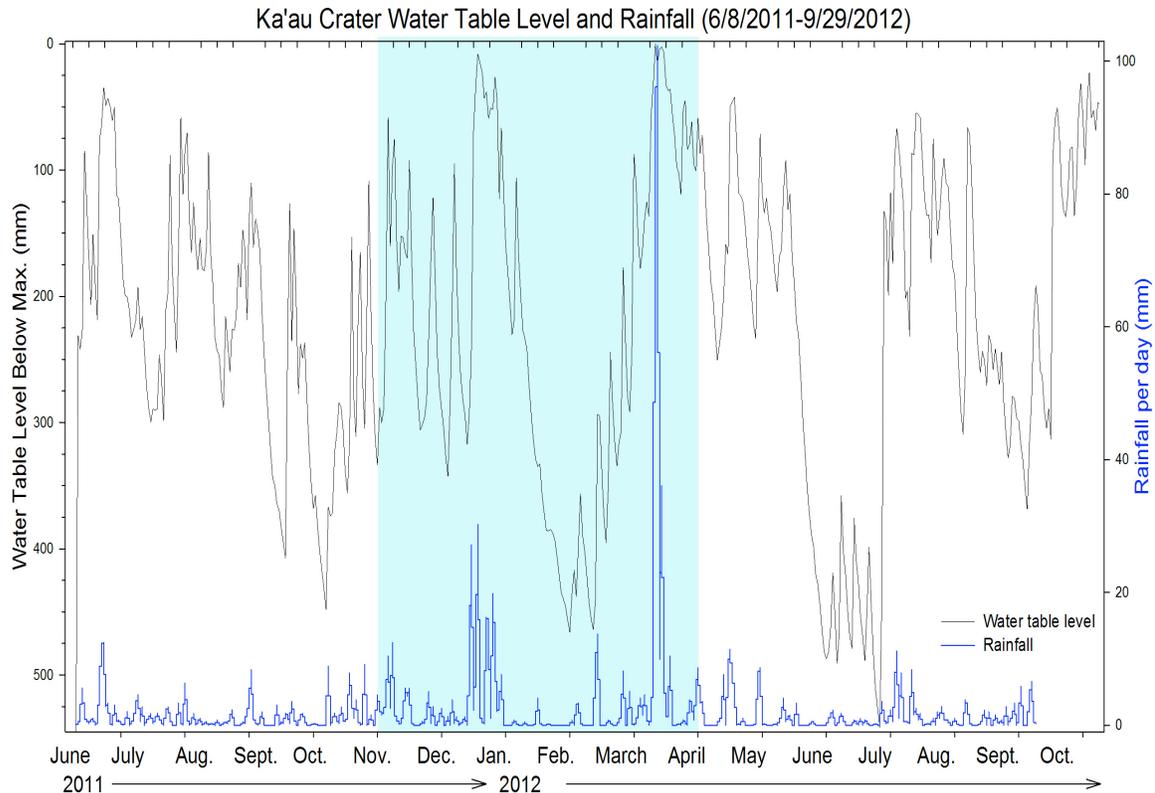


Figure 4. Rainfall in Palolo Valley (station GHCND:USC00517664) and changes in the position of the local water table in the center of the Kaau Crater peatswamp. The blue bar shows the Nov.–March rainy season in Hawaii (Chu and Chen, 2005. Monitoring continues at Kaau Crater to the present.

Future Funding

Data, analysis, and hypotheses generated from this project were the seed for a subsequent proposal, which was submitted to the Pacific Islands Climate Change Cooperative and the Pacific Islands Climate Change Center on 2 April 2012. Although this proposal was not recommended for funding, future proposals to continue our growing knowledge of past rainfall in Hawaii will use the data collected here as a foundation. For example, near-future proposal submissions are planned for NSF (Paleoclimate Perspectives on Climate Change program) and the Pacific Islands Climate Science Center. A small grant (\$5000) was received by undergraduate student Karl Hsu under the mentorship of PI Beilman that will continue the geochemical component of this work in 2013–2014.

Publications Cited in Synopsis

- Anderson, L., M.B. Abbott, B.P. Finney, S.J. Burns, 2005, Regional atmospheric circulation change in the North Pacific during the Holocene inferred lacustrine carbonate oxygen isotopes, Yukon Territory, Canada, *Quaternary Research*, 64, 21–35.
- Chu, P.-S., 1995, Hawaii rainfall anomalies and El Niño, *Journal of Climate*, 8, 1697–1703.

- Chu, P.-S., H. Chen, 2005, Interannual and interdecadal rainfall variations in the Hawaiian Islands, *Journal of Climate*, 18, 4796–4813.
- Giambelluca, T.W., Q. Chen, A.G. Frazier, J.P. Price, Y.-L. Chen, P.-S. Chu, J.K. Eischeid, D.M. Delparte, 2013, *Online Rainfall Atlas of Hawaii*, *Bulletin of the American Meteorological Society*, 94, 313–316, <http://rainfall.geography.hawaii.edu>.
- Giambelluca, T.W., 1993, Climate and groundwater, in M. Sanderson, ed., *Prevailing Trade Winds, Weather and Climate in Hawaii*, Honolulu, Hawaii, University of Hawaii Press.
- Grand, M., E. Gaidos, 2010, Methane emission from a tropical wetland in Kaau Crater, Oahu, Hawaii, *Pacific Science*, 64, 57–72.
- Moy, C.M., G.O. Seltzer, D.T. Rodbell, D.M. Anderson, 2002, Variability of El Niño/Southern Oscillation activity at millennial timescales during the Holocene epoch, *Nature*, 420, 162–165.
- Rooney, J., C. Fletcher, E. Grossman, M. Engels, M. Field, 2004, El Niño influence on Holocene reef accretion in Hawaii, *Pacific Science*, 58, 305–324.
- Uchikawa, J., B.N. Popp, J.E. Schoonmaker, A. Timmermann, S.J. Lorenz, 2010, Geochemical and climate modeling evidence for Holocene aridification in Hawaii: Dynamic response to a weakening equatorial cold tongue: *Quaternary Science Review*, 29:3057–3066, doi:10.1016/j.quatscirev.2010.07.014.

Student Support

Three students have been supported on this project; one Master's student (Ms. Olivia Schubert) and two undergraduate students (Mr. Rhys Ormond and Mr. Karl Hsu). These students have been supported by the Section 104 Base Grant. Mr. Karl Hsu is a member of the University of Hawaii's Honors Program, and is in the top 15% of all undergraduates on campus. Mr. Hsu recently received an Undergraduate Research Grant (\$5000) from the Undergraduate Research Opportunities Program at the University of Hawaii at Manoa. This funding will allow him to continue to analyze the first-ever core raised from Kaala (Figure 5) and also explore more sophisticated geochemistry, namely changes in lignin monomers in Kaala sediments. A *Notable Award* was received by Ms. Olivia Schubert at the 2011 Tester Symposium at the University of Hawaii at Manoa, where she received Best Student Poster for her presentation entitled "Long-term aspects of mountain rainfall and vegetation change, Oahu," which showcased early results from this project. Ms. Schubert is now employed as a full-time technician in the geochemistry lab of Dr. Hope Jahren of the Department of Geology and Geophysics at the University of Hawaii.

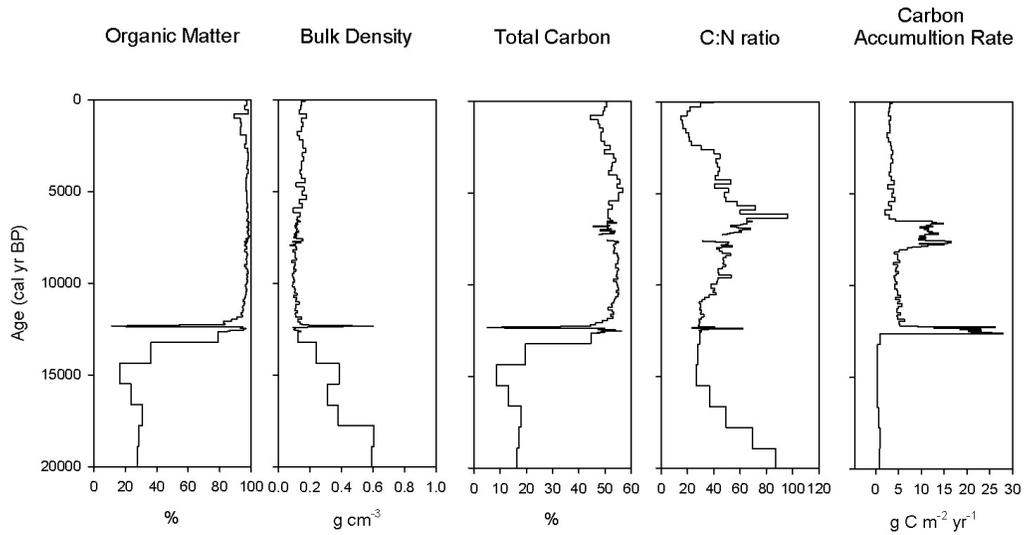


Figure 5. Preliminary data for physical and chemical properties of a 150-cm peat core raised from Ka'ala mountain. The age-depth relationship was derived from 5 radiocarbon dates using funding from other sources.

Development of an Advanced Surface Tensiometer for Measuring Water Quality

Basic Information

Title:	Development of an Advanced Surface Tensiometer for Measuring Water Quality
Project Number:	2011HI320B
Start Date:	3/1/2011
End Date:	2/28/2013
Funding Source:	104B
Congressional District:	Hawaii 1st
Research Category:	Not Applicable
Focus Category:	Water Quantity, None, None
Descriptors:	
Principal Investigators:	Yi Zuo

Publication

1. Xianju Wang, Remei Chen, Russell Valle, Zdenka Policova, A. Wilhelm Neumann, Yi Y. Zuo*, Melting of DPPC monolayer. (in preparation)

FINAL REPORT

**Development of an Advanced Surface Tensiometer
for Measuring Water Quality**

May 2013

Yi Zuo

Project Number: 2011HI320B

Water Resources Research Center
University of Hawaii at Manoa
Honolulu, Hawaii

Final Report (March 1, 2012 - February 28, 2013)

Development of an Advanced Surface Tensiometer for Measuring Water Quality

1. Problem and Research Objectives

Characterizing the physical, chemical and biological properties of potable and recreational waters plays a vital role in assessing and controlling water quality. Direct measurements of water quality mainly rely on (1) Physical assessment, such as pH, temperature, turbidity, and total dissolved solids; (2) Chemical assessment, such as salinity, dissolved oxygen, biochemical oxygen demand; and (3) Biological assessment, such as presence and abundance of microorganisms and insects. Due to the high-cost associated with direct measurements of water quality, ongoing monitoring programs are typically conducted by government agencies. Hawaii has more than 400 public beaches stretching along nearly 300 miles of Pacific Ocean coastline. According to the 20th annual beachwater quality report released by the Natural Resources Defense Council (NRDC) on July 28, 2010, pollution continues to contaminate the water at America's beaches, causing 2,352 closing and advisory days in Hawaii last year and 18,682 nationwide. *Therefore, there is an urgent need, especially for Hawaii, to develop an inexpensive, easy-to-use, and highly sensitive technique for measuring water quality.*

Surface tension of water is a physical property highly sensitive to contamination. A trace amount of pollutants (e.g., organic chemicals and microorganisms) can adsorb to the air-water interface, thus decreasing surface tension of pure water. *Therefore, surface tension measurement can be used as a novel and sensitive physical method to detect water quality.* Dynamic surface tension measurement has long been recognized as a means of evaluating water quality.¹ The adhesion and growth of marine bacteria have been found to depend on surface tension, and therefore, potentially have a direct impact on development of some diseases.² Compared to other physical, chemical, and biological methods for assessing water quality, surface tension is relatively easy to measure and hence may be an useful control parameter for water quality and water-reuse systems.²

The **objective** of this proposed project is to develop an advanced surface tensiometer for measuring water quality. This method has the potential to be developed into a powerful screening tool for assessing water quality and other environmental impacts of water contaminants.

2. Methodology

The proposed surface tensiometer is developed based on the principle of drop shape analysis.³ That is, in equilibrium, the shape of a drop or a bubble is determined by the balance between gravity, which tends to deform the drop (elongate a pendant drop or flatten a sessile drop), and surface tension force, which tends to hold the drop spherical. The force balance is determined by the Laplace equation of capillarity. If the shape of a

drop or bubble is known (e.g., by photographing or videotaping), it is possible to determine surface tension by solving the Laplace equation. The drop shape analysis offers a number of advantages as it requires less liquid sample, is applicable to both air–liquid and liquid–liquid interfaces, and is versatile and applicable to various situations.

Specifically, the proposed surface tensiometer is called the constrained drop surfactometer (CDS). As shown in **Fig. 1**, the CDS uses a small sessile drop ($\sim 10\text{--}20\ \mu\text{L}$) to measure the surface tension of liquid sample. Any surface active pollutant, such as ocean surfactant, is expected to adsorb at the air-water of the sessile drop to decrease surface tension of pure water. The specific physicochemical properties of the pollutant can be further characterized by measuring its surface rheological properties, in which the adsorbed film will be compressed and expanded by withdrawing liquid from and injecting liquid into the droplet using a motorized syringe. A key design of the CDS is a carefully machined drop holder which uses a sharp knife-edge to prevent the droplet from spreading even at very low surface tension (i.e., high surface pressure). In this case, the excess line energy of the sharp edge outweighs the weak surface tension in maintaining the integrity of the sessile drop. In addition, due to its compact design, the CDS allows accurate surface tension measurements with a controlled environment using a drop chamber.

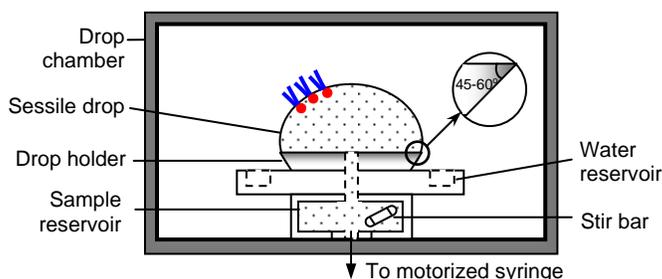


Figure 1. Schematic of the constrained drop surfactometer (CDS).

The surface tension of the liquid sample will be determined from the shape of the sessile drop using Axisymmetric Drop Shape Analysis (ADSA). ADSA is a patent-pending software package developed by the PI.^{4, 5, 6} ADSA features an optimized computational algorithm and an automatic image analysis scheme, thus permitting real-time and dynamic surface tension measurements.⁴ In addition to surface tension, ADSA simultaneously outputs surface area, drop volume, and curvature at the drop apex. All this information is valuable for characterizing properties of water samples. ADSA is superior to all existing commercial software packages in terms of rapid and highly accurate calculation, which is a key requirement for high-throughput screening. Meanwhile, ADSA features a user-friendly PC interface which allows surface tension measurement on one-click without the need of pre-training and knowledge of surface science. The applicability and accuracy of ADSA for measuring dynamic surface tension have been clearly demonstrated.^{4, 5, 6}

3. Principal Findings and Significance

3.1. Prototype Development

During the past 12-month period, we have successfully developed the prototype of the CDS. As shown in **Fig. 2**, the prototypes consists of three primary modules: the optical module, the liquid handling module, and the environmental control module. The optical module, which consists of a high resolution CMOS camera and a high-performance LED backlight, was developed with a separate grant. The liquid handling and the environmental control modules were developed in this project.

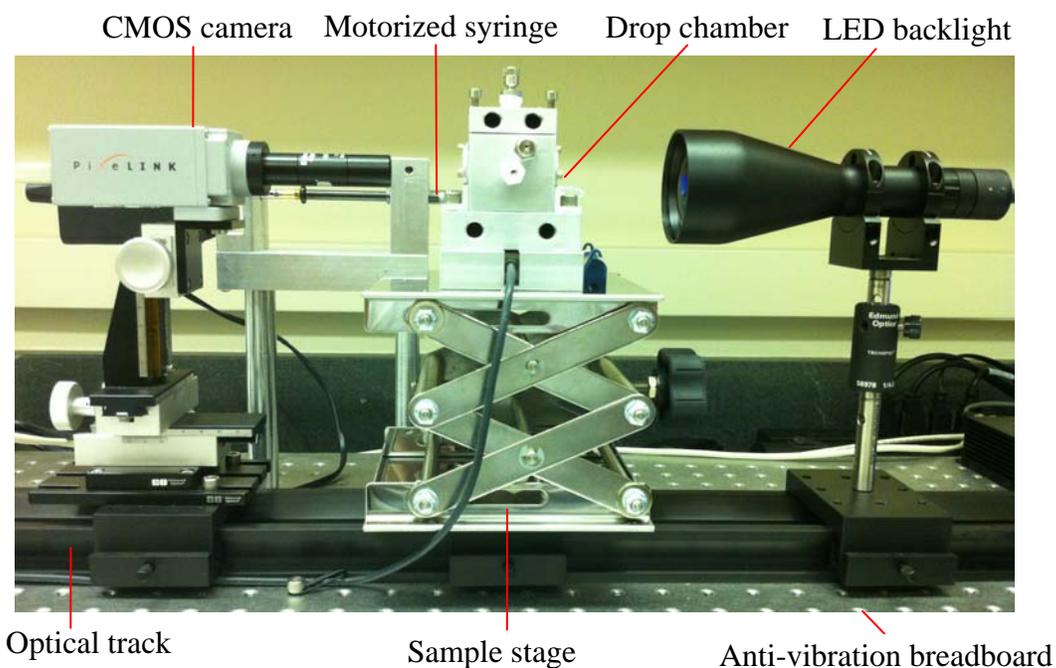


Figure 2. Prototype overview of the constrained drop surfactometer (CDS).

3.1.1. Liquid Handling Module

The liquid handling module was developed based on a motorized syringe. We have developed a LabVIEW program (**Fig. 3**) to precisely control movement of the motor, including the travel distance, rate, and fashion of movement (forward, backward, and cycling). This will allow us to automatically pump the liquid sample, form the droplet, and study the rheological properties of the liquid sample.

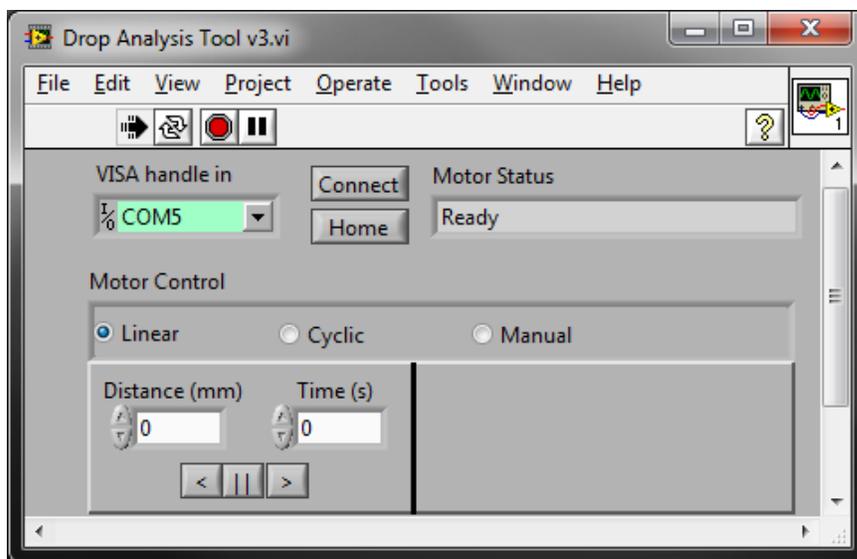


Figure 3. LabVIEW program to control the motorized syringe.

3.1.2. Environmental Control module

The environmental control module was developed based on a drop chamber (**Fig. 4**), designed and machined in the machine shop of the Department of Mechanical & Industrial Engineering at the University of Toronto. The temperature is controlled within ± 1 °C externally by a circulating water bath.

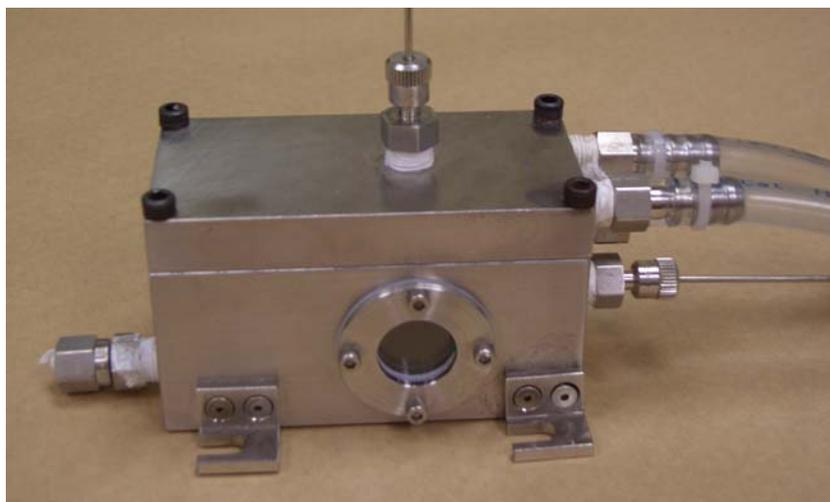


Figure 4. Drop chamber of the constrained drop surfactometer (CDS).

3.2. Test Results

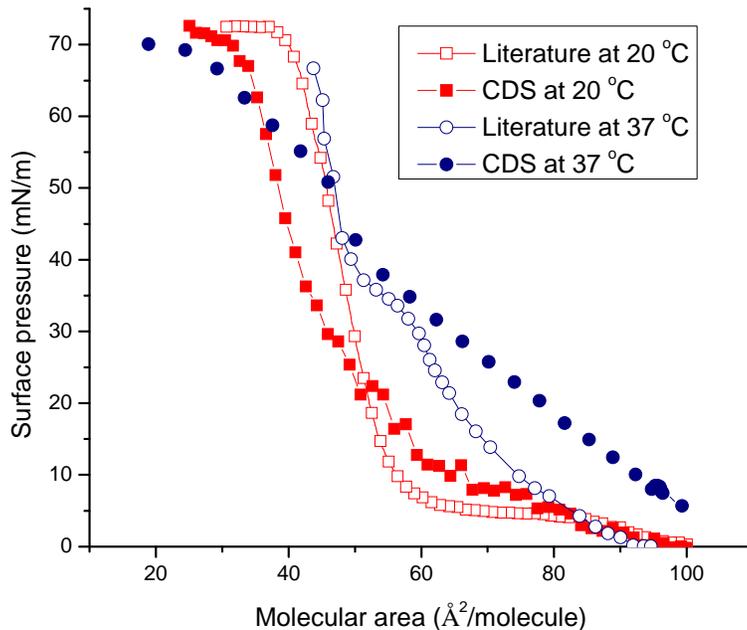


Figure 5. Comparison of DPPC isotherms at 20 °C and 37 °C obtained from the CDS to literature values obtained with established methods.

To test the CDS prototype, we have measured the surface pressure - surface area isotherms of dipalmitoyl phosphatidylcholine (DPPC) monolayers at the room and body temperatures. Surface pressure is defined to be the difference between the surface tension of pure water (~ 72 mN/m at room temperature) and the surface tension of film-covered (i.e., contaminated) water surface. Therefore, increasing surface pressure corresponds to decreasing surface tension. To verify our measurements, we have compared the isotherms obtained from the CDS prototype to those obtained with established methods. The standard DPPC isotherm at room temperature (20 °C) was obtained by the classical Langmuir balance.^{7,8,9} The DPPC isotherm at body temperature (37 °C) was produced by Crane and Hall using the captive bubble surfactometer (CBS).¹⁰ As shown in **Fig. 5**, at both temperatures, our measurements show some agreement with the literature values produced with the established methods. We will further develop our technique to increase its accuracy.

4. Publications Cited in Synopsis

1. Loglio G, Tesei U, Ficalbi A, Cini R. Dynamic surface tension measurements for the assessment of potable water quality. *Talanta* 1976, **23**(4): 339-341.
2. Colt J. Water quality requirements for reuse systems. *Aquacultural Engineering* 2006, **34**(3): 143-156.
3. Neumann AW, David R, Zuo Y (eds). *Applied Surface Thermodynamics*. CRC Press: Boca Raton, FL, 2010.
4. Zuo YY, Do C, Neumann AW. Automatic measurement of surface tension from noisy images using a component labeling method. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 2007, **299**(1-3): 109-116.
5. Zuo YY, Ding M, Li D, Neumann AW. Further development of Axisymmetric Drop Shape Analysis-Captive Bubble for pulmonary surfactant related studies. *Biochimica Et Biophysica Acta-General Subjects* 2004, **1675**(1-3): 12-20.
6. Zuo YY, Ding M, Bateni A, Hoorfar M, Neumann AW. Improvement of interfacial tension measurement using a captive bubble in conjunction with axisymmetric drop shape analysis (ADSA). *Colloids and Surfaces a-Physicochemical and Engineering Aspects* 2004, **250**(1-3): 233-246.
7. Zhang H, Fan Q, Wang YE, Neal CR, Zuo YY. Comparative study of clinical pulmonary surfactants using atomic force microscopy. *Biochimica et biophysica acta* 2011, **1808**: 1832-1842.
8. Klopfer KJ, Vanderlick TK. Isotherms of dipalmitoylphosphatidylcholine (DPPC) monolayers: Features revealed and features obscured. *Journal of colloid and interface science* 1996, **182**(1): 220-229.
9. McConlogue CW, Vanderlick TK. A close look at domain formation in DPPC monolayers. *Langmuir* 1997, **13**(26): 7158-7164.
10. Crane JM, Hall SB. Rapid compression transforms interfacial monolayers of pulmonary surfactant. *Biophys J* 2001, **80**(4): 1863-1872.

Reshaping the Regulatory Framework for Hawaii Aquaculture - Water Quality Standards, Coastal Fishponds, and Shellfish Grounds

Basic Information

Title:	Reshaping the Regulatory Framework for Hawaii Aquaculture - Water Quality Standards, Coastal Fishponds, and Shellfish Grounds
Project Number:	2011HI323B
Start Date:	3/1/2011
End Date:	2/28/2014
Funding Source:	104B
Congressional District:	Hawaii 1st
Research Category:	Water Quality
Focus Category:	Water Quality, Law, Institutions, and Policy, Management and Planning
Descriptors:	
Principal Investigators:	David C. Penn

Publication

1. Penn, D., and M. Coleman, 2012, "Protective measures for shellfish areas—Exploring new water quality standards, watershed management approaches, and closure criteria for Hawaii's coastal systems," 15th International Conference on Shellfish Restoration, Groton, CT, Dec. 12–15.

Problem and Research Objectives

Aquaculture has become an important food source for Hawaii's growing population; however, water quality regulations have not evolved to accommodate the needs of this growing industry. The goal of this project is to derive and disseminate possible revisions to water quality regulations, through a broad-based collaborative effort, that incorporates the considerable importance of the aquaculture industry while protecting and maintaining a healthy environment.

Research objectives that support the achievement of this goal are (1) identify potential water quality standards revisions; (2) document procedures and information needs for each type of revision; (3) analyze the revision process for a coastal fishpond; and (4) estimate the resources needed for wider-scale revisions.

Methodology

The regulatory framework for Hawaii aquaculture spans large realms of interlocking federal, state, and county authority. Through consultation with fishpond operators, shellfish growers, aquaculture scientists, and agency personnel, we are identifying and explaining the structure and mechanics of existing and potential measures for protecting and improving inland and coastal water quality, facilitating and expanding coastal fishpond operations, and establishing coastal shellfish grounds. Our efforts are grounded in technically based policy analyses, utilizing readily available regulatory information and scientific data.

Much of our work involves tracking and evaluating legislative and regulatory initiatives and the outcomes of judicial proceedings that affect the options available for Hawaii producers and their watershed partners. We are also studying comparable water quality standards and shellfish sanitation programs across the U.S. and throughout the world. Augmented by additional funding obtained through ongoing collaboration with Sea Grant investigators (Haws, 2012), the dissemination of our results will now include a geospatial mapping component that is integrated with emerging Sea Grant research products such as the Hawaii Aquaculture Digital Atlas and utilizes newly available interactive viewers such as the Hawaii Aquaculture Marine Mapper (<http://www.pifsc.noaa.gov/marinemapper>).

Principal Findings and Significance

The regulatory framework for Hawaii aquaculture has a lot of moving parts that do not always move in a coordinated fashion. Some of the most significant changes encountered during the project year include (1) proposed revisions to the state's methodology for making waterbody impairment decisions (Department of Health, 2012a); (2) revision of state administrative rules governing the application of pesticides in state waters (Department of Health, 2012b); (3) pending revision of state shellfish sanitation program regulations; and (4) development of a statewide programmatic general permit and programmatic agreement for the restoration, repair, maintenance, and reconstruction of traditional Hawaiian fishpond systems (Office of Conservation and Coastal Lands, 2012).

Through initial consultation with fishpond and shellfish researchers at the Pacific Aquaculture and Coastal Resources Center, University of Hawaii at Hilo, we learned of the difficulties faced by producers in meeting the water quality requirements established by the state shellfish sanitation program. Subsequent meetings and site visits with fishpond operators and agency personnel revealed both frustration with the lack of suitable controls on watershed processes (a cause of some of these difficulties) and determination to proceed with the testing necessary to obtain growing area classification and develop appropriate technical responses, such as the establishment of depuration facilities. We found that tools in other jurisdictions enable more proactive approaches to enhancing the water quality of fishponds and shellfish growing areas, such as State of Washington provisions for “shellfish protection districts” and “marine recovery areas” (Washington State Legislature, 2013).

A follow-up meeting with the staff of the state shellfish sanitation program improved our understanding of its plans and research needs. Our review of a preliminary draft of the pending new rules governing the state shellfish sanitation program revealed thorough consistency with National Shellfish Sanitation Program regulations and guidance. This would allow Hawaii shellfish that meet program specifications to be sold commercially and shipped to other U.S. destinations. However, resource constraints limit the scope of water quality testing that can be conducted to establish, maintain, and upgrade the necessary growing area classifications.

Through a meeting with federal and state aquaculture program administrators, we learned about a new federal-state-private initiative to streamline the permitting process for fishpond restoration activities. Although our proposal to conduct some of this work was not selected for funding (Minerbi, 2012), we will remain involved in the coming project year by (1) joining an upcoming water quality focus group meeting to develop justifications supporting the issuance of a blanket water quality certification for fishpond restoration activities; (2) reviewing a draft environmental assessment for the proposed state programmatic general permit; and (3) providing updates on our research findings.

Potential Water Quality Standards Revisions

Waterbody Type. State of Hawaii water quality standards (WQS) establish a suite of inland and marine waterbody types and marine bottom ecosystem types that form a basis for applying (1) discharge prohibitions, and (2) discrete sets of specific numeric criteria for evaluating various water quality and sediment quality parameters (Department of Health, 2012b). “Fishpond” and “shellfish grounds” are not among the named waterbody types established in the WQS, so they are generally regulated based on their site-specific hydrographic context. For example, a coastal fishpond with surface hydrologic connections to adjacent marine waters is regulated according to the adjacent marine waterbody type (either “embayment,” “open coastal,” or “Kona”).

However, open coastal water criteria are more conservative than embayment criteria (Kona criteria are site-specific and indexed to salinity gradients), and discharges that are prohibited in certain embayments are not prohibited in open coastal waters. This situation holds advantages and disadvantages for the siting and operation of fishponds and shellfish grounds, which could be reshaped in different ways, including revising the WQS to establish new waterbody types for “fishpond,” “shellfish grounds,” and adjacent, transitional marine waters. The establishment of such new, functionally-specific waterbody types would provide a basis for adopting associated, functionally-appropriate discharge prohibitions and evaluative criteria for both the new waterbody types and their contributing and receiving waters.

Protected Uses. The WQS in other jurisdictions formally assign use protection on a waterbody-specific basis. For example, California’s menu of twenty “beneficial uses” includes “Commercial and Sport Fishing,” Fish Spawning,” and “Shellfish Harvesting,” and a set of regional basin plans establish the specific waterbodies that are designated for the protection of each of these uses (e.g., San Francisco Bay Region, 2011). However, the Hawaii WQS establish “uses to be protected” in state inland and marine waters on a non-specific, class-by-class basis that (1) covers all waterbodies within a class and (2) ignores waterbody type and marine bottom ecosystem type distinctions and individual waterbody boundaries. For example, all marine waters, regardless of waterbody type (embayment, open coastal, Kona, and oceanic) and location, are either Class A or Class AA, and the specific numeric criteria established for evaluating each waterbody type do not vary by class and associated class-specific uses.

Water class, like waterbody type, also forms a basis for applying discharge prohibitions. For example, zones of mixing are prohibited in nearshore portions of Class AA waters, but are not prohibited in Class A waters. Although “the support and propagation of shellfish and other marine life” is an explicit “use to be protected” in Class AA waters, the WQS for Class A waters merely provide that “that their use for recreational purposes and aesthetic enjoyment be protected. Any other use shall be permitted as long as it is compatible with the protection and propagation of fish, shellfish, and wildlife, and with recreation in and on these waters.”

As with waterbody type, the combination of class-based discharge prohibitions and protected uses can cut both ways for fishpond and shellfish production interests, and could be reshaped in different ways. These include (1) the establishment of new, functionally-specific water classes that focus on use attainment for fishpond aquaculture and shellfish production, and (2) the explicit assignment of traditional and customary fishpond aquaculture and open water shellfish production as explicit uses to be protected in existing waterbodies, waterbody types, and water classes. A separate, more ambiguous, and potentially more limiting situation exists with regards to marine bottom ecosystem class, where class I “[u]...are passive human uses without intervention or alteration...,” and on class II marine bottoms, “[a]ny action which may permanently or completely modify, alter, consume, or degrade marine bottoms” requires a myriad of approvals.

Biological Monitoring and Assessment Methodologies. The existing regulatory framework does not provide explicit monitoring approaches and methodologies for assessing whether or not “the support and propagation of shellfish and other marine life” is fully attained in state waters. Such decisions generally occur in a case-by-case permitting context, where agency discretion and professional judgment is applied to “determine parameters, measures, and criteria for bottom biological communities which may be affected by proposed actions,” and “water quality standards [are] deemed to be met if time series surveys of benchmark stations indicate no relative changes in the relevant biological communities, as noted by biological community indicators or by indicator organisms which may be applicable to the specific site.” Although there is a well-established program for monitoring and evaluating coral reef health on a statewide basis (Rodgers et al., 2010), the program’s assessment methods have not been adopted by the State of Hawaii Department of Health (DOH) for evaluating attainment of the WQS, and similar programs and methods have not been developed for other marine bottom ecosystem types. Therefore, in addition to challenges posed by sanitation program requirements, the re-establishment of historic shellfish grounds and the establishment of new shellfish grounds in nearshore marine waters would appear to require intensive documentation of existing bottom conditions and justification of proposed changes thereto.

Procedures and Information Needs for Revisions

Potential revisions to the water quality standards that would support the advancement of Hawaii aquaculture include (1) changing the framework of waterbody and marine bottom types and classes; (2) establishing and assigning designated uses (uses to be protected) on a more site-specific basis; (3) developing evaluative criteria that are explicitly tied to specific types, classes, uses, and sites; and (4) developing and implementing biological monitoring and assessment methods that are directly linked with use attainment decisions. Mechanisms for initiating such regulatory change are available for agencies, elected officials, and citizens through a variety of legislative and administrative processes, but will usually require agency cooperation to be successful.

For example, although a party may petition the DOH to initiate specific changes to the WQS (Department of Health, 2005), there is no guarantee that (1) the department will grant the petition, (2) the requested changes will be incorporated into a formal departmental proposal, and (3) the governor and the U.S. Environmental Protection Agency (EPA) will approve the department's proposal. Similarly, even though a party may succeed in introducing legislation that enacts revisions to the WQS (State of Hawaii, 2009), the DOH still decides whether or not to submit the revisions for EPA approval. Therefore, more efficient and productive solutions may exist in targeting aquaculture activity zones for proactive, partnership-based watershed improvements.

Revision Process for Coastal Fishponds

During the project year, the Hawaii Coastal Zone Management (CZM) Program consulted with the U.S. Army Corps of Engineers about a proposed program change that would provide general CZM concurrence for minor federal permit activities for Hawaiian fishpond restoration, repair, maintenance, and reconstruction. To complement this effort and the permit streamlining currently being developed by the Hawaii Department of Land and Natural Resources, we are constructing a matrix of fishpond regulatory settings that defines the range of existing regulatory frameworks involved, including:

- Waterbody type (marine open coastal waters, marine embayment, inland estuary, inland wetland)
- Marine bottom ecosystem type (sand beach, lava rock shoreline/solution bench, marine pool/protected cove, artificial basin, reef flats/communities)
- Water class (AA/A marine water; 1(a)/1(b)/2 inland water, I/II marine bottom)
- Class AA marine waterbody subtype (zone of mixing prohibited)
- Adjacent to a sandy beach (triggers environmental review requirements for restoration activities)

This matrix will provide coding that can be added to an existing fishpond inventory (DHM Planners, Inc., 1989, 1990), provide a key for displays in resource mapping applications, and be employed by interested parties to identify and analyze potential options for site-specific regulatory reform.

Resource Needs for Wider-Scale Revisions

The results of our dialogue with all parties suggest that the State of Hawaii does not have sufficient resources to support the management of extensive nearshore shellfish growing grounds. Limited potential for large-scale, commercially-viable enterprises makes it unlikely that industry would fund the development and operation of management capacity as it has done in other states. However, momentum is growing and significant accomplishments are pending that would greatly improve the regulatory framework for fishpond aquaculture. There is great hope for the restoration of shellfish as food supply in Hawaii based on (1) closed systems that use groundwater sources which more easily achieve the water quality requirements for growing area approval, and (2) cultivation in fishponds where public access can be limited and management efforts intensified. If the repair of inland ecosystems and the improvement of watershed health is aggressively promoted and adequately supported, the water quality of fishponds and other shellfish growing grounds may eventually become sufficient to allow for fully approved areas that require less stringent management, and conditionally approved areas that can be serviced by nearby depuration facilities.

Publications Cited in Synopsis

- California Regional Water Quality Control Board San Francisco Bay Region, 2011, Chapter 2: beneficial uses, in *San Francisco Bay Basin (Region 2): Water Quality Control Plan (Basin Plan)*, Office of Administrative Law, State of California, http://www.waterboards.ca.gov/rwqcb2/water_issues/programs/planningtmdls/basinplan/web/bp_ch2.shtml.
- Department of Health, 2005, Rules of Practice and Procedure, in *Hawaii Administrative Rules*, Chapter 11-1, State of Hawaii, http://hawaii.gov/health/about/rules/index.html/prac_proc.pdf.
- Department of Health, 2012a, 2012 State of Hawaii water quality monitoring and assessment report: Integrated Report to the U.S. Environmental Protection Agency and the U.S. Congress Pursuant to §303(d) and §305(b), Clean Water Act (P.L. 97-117) (draft), Honolulu, Hawaii, <http://hawaii.gov/health/about/admin/health/environmental/water/cleanwater/index.html>.
- Department of Health, 2012b, Water quality standards, in *Hawaii Administrative Rules*, Chapter 11-54, State of Hawaii, <http://hawaii.gov/health/about/admin/health/environmental/water/cleanwater/index.html>.
- DHM Planners, Inc.; Bernice Pauahi Bishop Museum; Moon, O'Connor, Tam & Yuen; 1990, Hawaiian fishpond study: islands of Hawaii, Maui, Lanai and Kauai; Honolulu, Hawaii, DHM Inc.
- DHM Planners, Inc., Hawaii Office of State Planning, Bernice Pauahi Bishop Museum, 1989, Hawaiian fishpond study: islands of Oahu, Molokai, and Hawaii; Honolulu, Hawaii, DHM Planners.
- Haws, M., 2012, Expanding and diversifying near-shore mariculture in Hawaii and the U.S. Pacific Islands through resolution of regulatory, technical, and biological impediments, (national Sea Grant aquaculture research project awarded in 2012; D. Penn, key personnel), http://www.seagrant.noaa.gov/funding/2012_National_Sea_Grant_Aquaculture_Projects.pdf.

- Minerbi, L., 2012, Development of an environmental assessment and state programmatic general permit for the maintenance, repair, reconstruction, and operation of fishponds in Hawaii, (proposal submitted to Conservation International by the University of Hawaii at Manoa; R. Babcock, D. Penn, co-investigators).
- Office of Conservation and Coastal Lands, 2012, Status update of the streamlined permit process for Hawaiian fishponds, report to the Twenty-Seventh Legislature, 2013 Regular Session, Department of Land and Natural Resources, State of Hawaii, <http://files.hawaii.gov/dlnr/reports-to-the-legislature/2013/OCCL13-Streamedline-Fish-Pond-Permit-Rpt.pdf>.
- Rodgers, K.S., P.L. Jokiel, C.E. Bird, E.K. Brown, 2010, Quantifying the condition of Hawaiian coral reefs, *Aquatic Conservation: Marine and Freshwater Ecosystems*, 20(1), 93–105.
- The Senate, Twenty-Fifth Legislature, State of Hawaii, 2009, SB1008 SD1 HD2 (Relating to Water Quality Standards), http://www.capitol.hawaii.gov/Archives/measure_indiv_Archives.aspx?billtype=SB&billnumber=1008&year=2009.
- Washington State Legislature, 2013, Revised Code of Washington, Chapters 90.72.030; 90.72.040(1); 70.118.A.020; and 70.118.A.040, <http://apps.leg.wa.gov/rcw/default.aspx?Cite=90.72>; <http://apps.leg.wa.gov/rcw/default.aspx?cite=70.118A>.

Addressing Sewage Contamination of Nawiliwili Streams and Kalapaki Beach

Basic Information

Title:	Addressing Sewage Contamination of Nawiliwili Streams and Kalapaki Beach
Project Number:	2011HI328B
Start Date:	3/1/2011
End Date:	2/28/2014
Funding Source:	104B
Congressional District:	Hawaii 1st
Research Category:	Not Applicable
Focus Category:	Water Quality, Surface Water, Non Point Pollution
Descriptors:	
Principal Investigators:	Marek Kirs

Publications

There are no publications.

Introduction

Problem

Problem One. USEPA requires every state to use USEPA approved methods to assay water samples to meet drinking water and recreational water quality standards. When these water quality standards are exceeded, USEPA directives conclude that the water samples are contaminated with sewage. However, extensive studies originally conducted by Water Resources Research Center (WRRC) at the University of Hawaii, and confirmed by research microbiologists throughout the nation, have shown that the data obtained are suggestive but do not confirm for the presence of sewage contamination. This problem is most apparent in the state of Hawaii because studies conducted by WRRC have shown that the fecal indicator bacteria (FIB), which are used to establish water quality standards, grow naturally in the soil environments of Hawaii. Therefore, the presence of FIB in Hawaii's environmental waters is more likely due to soil contamination than sewage contamination. Additional studies by WRRC laboratories have shown that analyzing water samples for alternative microorganisms found in sewage provide more reliable data for the presence and absence of sewage contamination.

Problem Two. A microbiologist will be hired and trained to replace R. Fujioka (retired WRRC microbiologist). The laboratories previously used by Fujioka will now be consolidated to the WRRC Analytical Laboratory (located in Holmes Hall). Fujioka will train the new WRRC microbiologist in the use of established and new methods so that all the necessary methods can be implemented in the WRRC Analytical Laboratory. The microbiologist will also be introduced to Hawaii's water agencies (Department of Health, state of Hawaii; City and County of Honolulu, Division of Wastewater; Honolulu Board of Water Supply) and will continue to assist them with their microbial water quality issues.

Problem Three. Fujioka completed a research study on the island of Kauai's Nawiliwili watershed where cesspools are extensively used. The conclusions of this study are as follows: 1) Many of the microbial water monitoring data obtained from Kauai were similar to data previously obtained on Oahu. 2) FIB (fecal coliforms, enterococci) are naturally present in high concentrations in soil and represent an environmental, non-sewage source of FIB. 3) Under ambient conditions, concentrations of FIB in streams routinely exceed current water quality standards and the predominating source of FIB is soil rather than sewage. 4) FIB are unreliable indicators of fecal contamination for streams and coastal waters receiving land based discharges on Kauai and Oahu. 5) Monitoring for F+ coliphages provided reliable data to detect subsurface contamination of streams by cesspool waste. 6) Although *C. perfringens* was previously shown to be a good indicator of surface sources for sewage pollution on Oahu, this fecal bacteria was not a good indicator for subsurface contamination of streams by cesspool waste. 7) Identifying and genotyping FRNA coliphages recovered from environmental water samples provided additional data to determine human or animal sources of fecal contamination. 8) The detection of elevated levels of FRNA coliphages of human sources (genotypes II, III) in streams on Kauai indicate that these streams are contaminated with cesspool waste and are likely to be contaminated with human sewage-borne viruses.

Objectives

A research project will be conducted to characterize the issues raised in the pollution of Kalapaki Beach by discharges of cesspool wastes into the Nawiliwili watershed.

The specific objectives of this project were stated as follows:

- 1) **Training.** The new WRRC microbiologist will be trained in water quality testing methods that assay for culturable levels of fecal indicator bacteria (enterococci, *E. coli*), as well as alternative fecal indicators (*C. perfringens*, coliphages) that are specifically associated with human sewage. The WRRC microbiologist will also be trained to become proficient in molecular genetic methods for human specific *Bacteroides* bacteria and for human specific enteric viruses that includes noroviruses.
- 2) **Internal collaboration.** The microbiologist will work with WRRC personnel (e.g., Mr. Joseph Lichwa and Mr. Philip Moravcik) to familiarize himself with WRRC's operations and to prepare proposals for research projects.
- 3) **External collaboration.** The microbiologist will be introduced to key personnel in the Hawaii State Department of Health, the City and County of Honolulu, and the Honolulu Board of Water Supply for the purpose of understanding their water quality issues and to submit grants to these agencies as well as national agencies to aid in solving the water quality problems facing the United States as well as foreign countries.

Methodology

A total of 117 water samples were collected by Gary Ueunten (Kauai Branch of HIDOH) from ten sites at the Nawiliwili watershed and Kalapaki Beach located at the eastern section of Kauai (Figure 1), as well as from one additional relatively pristine control site at the Lawai Stream located in the middle section of Kauai. Samples were collected roughly fortnightly from June to December 2011. After collection, samples were cooled on ice and shipped by air to the microbiology laboratory at WRRC. All samples were analyzed within 6–9 h after sample collection for FIB (enterococci, *E. coli*) and alternative fecal indicators (*C. perfringens*, coliphages). Every second sampling and subsamples were provided for Dr. Lu's laboratory at the University of Hawaii at Manoa for the analyses of noro-, entero-, and adenoviruses by polymerase chain reaction (PCR) based molecular tests. An additional set of membrane filters (PC and HA) was stored for molecular analysis at WRRC.

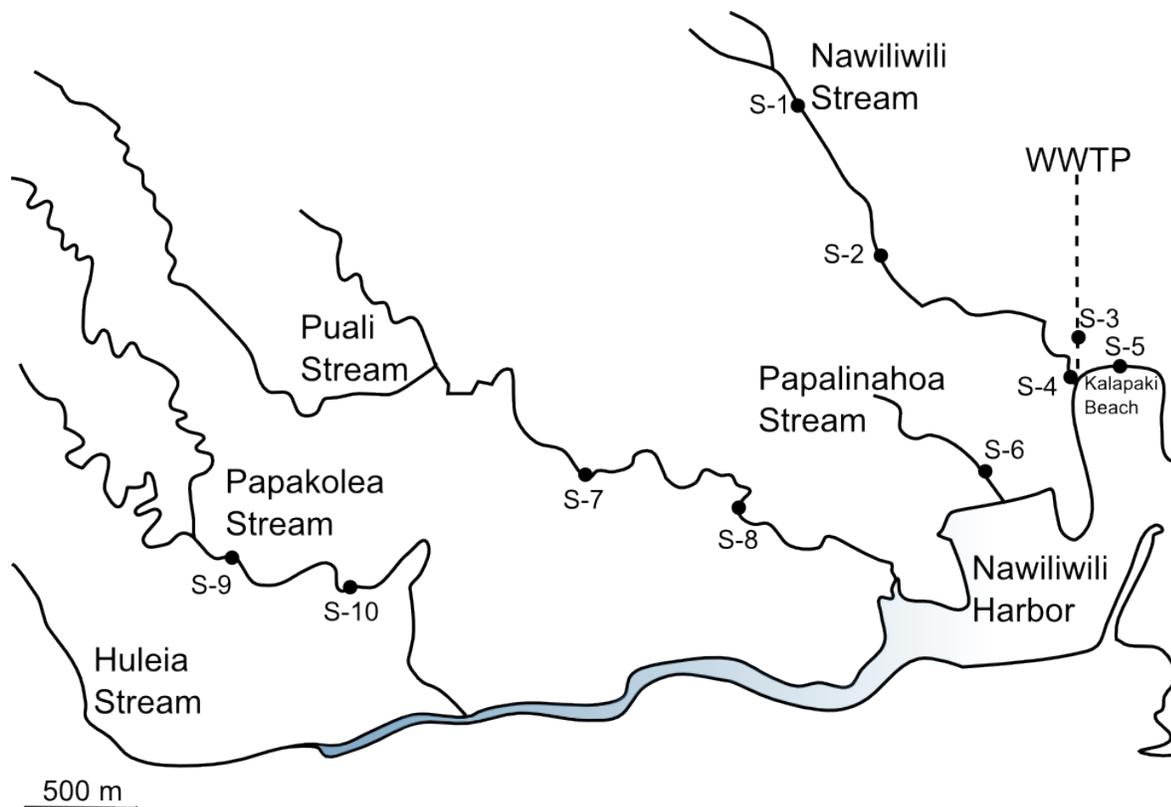


Figure 1. Nawiliwili watershed sampling sites: S-1 Upper Nawiliwili Stream, S-2 Lower Nawiliwili Stream, S-3 Mariott Culvert, S-4 Pine Trees, S-5 Kalapaki Beach, S-6 Papalinhua Stream, S-7 Upper Puali Stream, S-8 Lower Puali Stream, S-9 Upper Papakolea Stream, and S-10 Lower Papakolea Stream.

Concentrations of enterococci and *E. coli* were determined using USEPA approved membrane filtration based methods 1106.1 [1] and 1103.1 [2], respectively. In the case of enterococci the samples were filtered through a glass fiber filter (Pall Corporations, Ann Arbor, MI), the filter was placed on a mE agar plate and incubated at 41°C for 48 h. After incubation filters were transferred to pre-warmed EIA plates and incubated for an additional 20 min. Colonies with black or reddish brown precipitate on the underside of the membrane were counted as enterococci. In the case of *E. coli*, the samples were filtered through a glass fiber filter (Pall Corporations, Ann Arbor, MI), the filter was placed on mTEC agar plate and incubated first at 35°C for 2 h and then in a waterbath set at 44.5°C for another 22–24 h. After incubation the membranes were transferred to an absorbent pad saturated with Urea Substrate Medium for 15–20 min. Yellow, yellow-brown and yellow-green colonies were counted as *E. coli*.

Concentrations of *C. perfringens* spores were determined after incubation of 100 ml subsamples for 15 min at 60°C to kill the vegetative cells using SFP media based Fung double tube (FDT) test [3] during first sampling events. Due to the high background signal originating from other species of *Clostridium* in freshwater samples, conventional mCP agar based method [4] was used for the rest of the samples. After incubation of 24 h in an anaerobic chamber at 45°C for 24 h, the membranes were exposed to ammonium hydroxide fumes for 20 sec and pink colonies were counted as *C. perfringens*. Concentrations of somatic and male (F^+) specific coliphages were identified in 5 ml sample portions using USEPA approved Method 1601 [5] using *E. coli* CN-13 and *E. coli* F_{amp} as a host, respectively. Negative samples were assayed by enrichment using 100 ml sample portions the following day.

Molecular tests for human Bacteroides and polyomavirus markers, as well as *C. perfringens* and appropriate controls, were also setup and validated at WRRC. The process was delayed due to permits required for the control organisms. Quantification standards were setup based on the extracted quantified genomic DNA from control organisms or in the case of human polyomavirus, based on the cloned synthesized product, which was extracted, linearized and quantified. All standards were quantified on Qbit 2.0 lumionometer (Life Technologies). All quantitative PCR reactions were run on CFX96 real-time system (BioRad). DNA was extracted from control organisms as well as from the environmental samples (filter concentrated) using a PowerSoil DNA isolation kit (MoBio Laboratories).

Concentration of total phosphorus (PO_4^{3-}) was identified using acid persulfate digestion (method 8190) on Chemical Oxygen Demand reactor (Hach, Loveland, CO). Temperature, pH, salinity, and weather conditions were recorded in the field by HIDOH staff.

Principal Findings and Significance

All freshwater sites, this includes all sampling sites except Kalapaki Beach (which was a marine site, salinity: 29–34 ppm), were characterized by high concentrations of conventional indicator bacteria (*E.coli* and enterococci) throughout the study period. Concentrations of *E. coli* varied from <4 to 7920 CFU per 100 ml with a geometric mean varying from 196 to 1260 CFU per 100 ml between sites and concentrations of enterococci varied from 41 to 6040 CFU per 100 ml with a geometric mean varying from 76 to 1928 CFU per 100 ml between sites. Hence, concentrations of indicator bacteria were elevated, except at the marine site (S-5 Kalapaki Beach) where concentrations of enterococci were relatively low (4–26 CFU per 100 ml, with a geometric mean of 9 CFU per 100 ml) (Table 1). These results are comparable to the earlier study by Vithanage et al. [6], although no elevated fecal indicator bacteria concentrations were detected at the marine site.

Current Hawaii recreational freshwater water quality standards are based on enterococci and explicitly states that no sample should exceed 89 CFU of enterococci per 100 ml and geometric mean of samples collected over 25–30 days should not exceed 33 CFU of enterococci per 100 ml [7]. All samples collected, including the control sample from the pristine environment, exceeded the standard based on the geometric mean throughout the study period. The standard based on the single sample maxima was also exceeded in all freshwater samples, except for one sample collected from the Puali Stream and three out of four samples collected at the pristine control site (Lawai Stream). These findings collectively indicate that **current water quality standards are not suitable for Hawaii due to high environmental background of indicator bacteria**, and therefore have little relevance to the actual health risk.

Current Hawaii recreational marine water quality standards are based on enterococci and explicitly states that no sample should exceed 104 CFU of enterococci per 100 ml and geometric mean of samples collected over 25–30 days should not exceed 35 CFU of enterococci per 100 ml [7]. Samples collected at the Kalapaki Beach did not exceed the standards during the study.

Table 1. Geometric means of *E.coli*, enterococci, and alternative indicators at Nawiliwili watershed sampling sites. Minimum and maximum concentration are indicated in parenthesis.

Sampling Site	<i>E. coli</i> (CFU/100 ml)	Enterococci (CFU/ 100 ml)	<i>C. perfringens</i> (CFU/100 ml)	Somatic coliphages (PFU/100 ml)	Male (F⁺) coliphages (PFU/100 ml)
S-1	286 (4-1560)	255 (120-480)	3 (<1-25)	32 (<1-240)	2 (<1-40)
S-2	1151 (640-2040)	900 (480-1880)	4 (<1-30)	108 (20-440)	8 (<1-260)
S-3	239 (<4-7280)	780(284-2880)	4 (<1-15)	123 (20-1040)	7 (<1-160)
S-4	409 (<4-1440)	520 (256-1040)	4 (<1-10)	272 (80-2680)	11 (<1-260)
S-5	7 (<4-106)	9 (4-26)	3 (<1-15)	2 (<1-40)	1 (<1)
S-6	1050 (144-7920)	1928 (1000-3640)	6 (<1-56)	289 (<1-2620)	2 (<1-500)
S-7	475 (92-5440)	204 (88-480)	2 (<1-25)	87 (20-800)	2 (<1-140)
S-8	1260 (560-3920)	1113 (332-6040)	4 (<1-15)	78 (<1-800)	1 (<1-20)
S-9	352 (40-1120)	348 (128-2040)	3 (<1-35)	214 (60-600)	11 (<1-140)
S-10	741 (280-5600)	524 (240-1040)	4 (<1-15)	101 (<1-360)	4 (<1-40)
Lawai Stream (Control)	196 (99-305)	76 (41-140)	1 (<1-2)	18 (<1-140)	9 (<1-260)

C. perfringens concentrations were low during the study at all sites. No sample exceeded 50 CFU per 100 ml limit, except one sample collected at Papalinaloa Stream after wet weather. No spores of *C. perfringens* were found at the marine site (Kalapaki Beach). *C. perfringens* concentrations were low likely due to the prevailing dry weather pattern, although relatively large in size compared to pathogenic viruses that can further hamper transport of this bacterium through the soils [6].

Concentrations of somatic coliphages were comparable to the estimates in an earlier study (geometric mean 2–289 PFU per 100 ml). The same holds true for male (F⁺) specific coliphages (geometric mean 1–11 PFU per 100 ml), although concentrations were roughly 10 fold lower in Papakolea Stream (S-9 and S-10). Somatic coliphages were more frequently recovered and at higher concentrations when compared to (F⁺) specific coliphages. While somatic coliphages may multiply in the environment, (F⁺) specific coliphages cannot replicate in the environment as F⁺ pili are not formed at or below 25°C [8] and therefore (F⁺) coliphages could be good indicators of fecal contamination (animal and/or human). Therefore, based on the low concentrations of (F⁺) specific coliphages, fecal contamination appears to be limited in the study area. Although it should be considered that only a small percentage of humans and animals carry this group of phages, hence a leaking cesspool from a single family household or input from a small population of animals could remain undetected.

Tests for human viruses were conducted at six sites (S-2, S-3, S-4, S-6, S-8, and S-10) by Dr Lu's lab. Human norovirus genogroup 1, genogroup 2, enteroviruses, and adenoviruses were detected at all sites, except norovirus genogroup which was not detected at site S-10. While human adenoviruses were detected in all tested samples, a signal from other groups was inconsistent indicating fluctuating levels of sewage input. At this time, it is not clear whether the signal originates from viable viruses and what the associated health risk is, although detection of molecular signal originating from human viruses is an indication that **human sewage, likely from adjacent cesspools, is reaching the watershed.**

Inconsistent detection of human viruses in apparently pristine waters in the upper reaches of the watershed is intriguing. It is possible that some primers used in those tests may exhibit cross-reactivity with environmental material. This would not be surprising considering limited validation and end-point PCR based protocols used. Therefore, a completely new set of highly specific and quantitative source tracking tests is being set up and validated at WRRC. These test target human Bacteroides group (novel version based on HF183) and highly specific human polyomaviruses use probe based DNA amplification and quantification techniques. At this time, we have obtained the required permits, set up the molecular tests (standards and controls) as well as extracted DNA from all the 117 water samples. These samples are currently being analyzed and findings will be included in the final report.

Within the framework of this project, Dr. Kirs was introduced to Mr. Joseph Lichwa (WRRC, University of Hawaii at Manoa) and Mr. Philip Moravcik (WRRC, University of Hawaii at Manoa). Furthermore, collaboration with Dr. Lu (Office of Public Health Studies, University of Hawaii at Manoa) was established within this project. Dr. Kirs was introduced to Mr. Watson Okubo (Section Chief, Clean Water Branch, HDOH), Mr. Ross Tanimoto (Deputy Director, Department of Environmental Services, CCH), Mr. Ken Tenno (Laboratory Director, Laboratory Branch, Department of Environmental Services, CCH) after his arrival at WRRC.

Dr. Mayee Wong, a postdoctoral researcher, was hired in January 2013 partially from the funds of this project. She has been trained in all the methods used in this study and will continue to support this project.

The study can be extended to other watersheds depending on the stakeholders needs. Methods and tools validated in this study can be used and transferred to our partner laboratories (DOH, CCH) as needed. Most importantly, we envision that this study can support our NOAA and Sea Grant funding proposals as well as gain further interest and support from the DOH and CCH once molecular tests are completed.

Publication Cited in Synopsis

1. USEPA, 2002, Method 1106.1: “Enterococci in water by membrane filtration using membrane Enterococcus esculin iron agar (mE-EIA),” U.S.E.P.A., Editor, Office of Water, Washington D.C., Washington, p. 12.
2. USEPA, 2002, Method 1103.1: “*Escherichia coli* (*E. coli*) in water by membrane filtration using membrane-thermotolerant *Escherichia coli* agar (mTEC),” U.S.E.P.A., Editor, Office of Water, Washington, D.C., Washington, p. 19.
3. Fung, D.Y.C., R. Fujioka, K. Vijayavel, D. Sato, D. Bishop, 2007, “Evaluation of Fung double tube test for *Clostridium perfringens* and easy phage test for F-specific RNA coliphages as rapid screening tests for fecal contamination in recreational waters of Hawaii,” *Journal of Rapid Methods and Automation in Microbiology*, 15(3): 217–229.
4. Emerson, D.J., and V.J. Cabelli, 1982, “Extraction of *Clostridium perfringens* spores from bottom sediment samples,” *Applied and Environmental Microbiology*, 44(5): 1144–9.
5. USEPA, 2001, Method 1601: “Male-specific (F+) and somatic coliphage in water by two step enrichment procedure,” United States Environmental Protection Agency, Office of Water. Washington D.C., p. 1–32.
6. Vithanage, G., R. Fujioka, and G. Ueunten, 2001, “Innovative strategy using alternative fecal indicators (F⁺ RNA/Somatic Coliphages, *Clostridium perfringens* to detect cesspool discharge pollution in streams and receiving coastal waters within a tropical environment,” *Marine Technology Society Journal*, 45(2): 101–111.
7. HDOH, 2009, Amendment and Compilation of Chapter 11-54 Hawaii Administrative Rules in Water Quality Standards: Honolulu, Hawaii, p. 89.
8. Novotny, C.P., and K. Lavin, 1971, “Some effects of temperature on the growth of F pili,” *Journal of Bacteriology*, 107(3): 671–82.

Island Director's workshop/conference

Basic Information

Title:	Island Director's workshop/conference
Project Number:	2011HI330B
Start Date:	3/1/2011
End Date:	2/28/2014
Funding Source:	104B
Congressional District:	Hawaii 1st
Research Category:	Not Applicable
Focus Category:	None, None, None
Descriptors:	
Principal Investigators:	Chittaranjan Ray, Aly I El-Kadi, Philip Moravcik

Publications

There are no publications.

Island Director's Workshop/Conference

An effort was made to publish the conference proceedings for selected presentations made at the conference. However, a number of book publishers that were contacted declined, mostly due to the relatively narrow audience of specialized conference proceedings. Effort is underway to publish the proceedings as a Water Resources Research Center's publication. The selected authors will be contacted about submitting an updated version of their contributions.

Tentative Title

Water Resource Sustainability Issues on Tropical Islands

Editor

Aly I. El-Kadi

Overview

This volume will include selected papers presented at the Water Resource Sustainability Issues on Tropical Islands conference held in Honolulu, Hawaii, November 14–16, 2011. The issue of sustainability is especially critical for islands due to resource limitation and water vulnerability to contamination. The ever-increasing and competing demands include water supply to urban and rural communities, tourist facilities, industry, and farm animals. Additional non-consumptive uses include hydropower generation, navigation, and recreation. Further, alternative energy sources, such as bio-energy, have added more strain on water resources. Demands are multiplying due to population growth and urbanization. In some cases, water supplies are unable to deliver water on a 24-hour basis due to high leakage and sometimes wastage. The issues related to the coordinated management of surface water and groundwater are of prime importance. Water resources are particularly sensitive to climate change due to islands' particular nature. Water scarcity and vulnerability to drought, flooding, and other natural disasters considerably increase as island size decreases. Major factors affecting water resources include physical island characteristics, such as size and topography, climate, and human impact. Climate change can lead to further degradation of water quality, which is already a major problem in many islands. Contamination originates from point and non-point sources. Pollution sources include discharges of untreated or partially treated wastewater and animals farms, inadequate solid waste disposal sites, agricultural chemicals, leakage of petroleum products and toxic chemicals, sediment erosion, and saltwater intrusion. The small size and steep slopes of catchments on high islands enable water and pollutants to move quickly to downstream areas. The highly permeable soils and shallow water tables on small coral islands facilitate the rapid migration of pollutants to the subsurface. The reversal of these negative impacts is difficult and time consuming. Pollution affects human health due to microbiological contamination and elevated chemical levels in water supplies. High turbidity and suspended solids are experienced by consumers after periods of heavy rainfall. The effectiveness of water supply intakes and treatment systems is compromised by high-suspended sediment loads, leading to higher costs of providing clean, safe water supplies. Sedimentation in water supply reservoirs and rivers lead to disturbances in upstream catchments. Finally, sediments, bacteria, and chemicals are negatively affecting riverine and coastal environments. The conference presentations, addressing the issues outlined above, are grouped in sessions covering wastewater, flooding, climate, water supply and management, groundwater recharge, surface water and groundwater quality, water for energy, and submarine water discharge. Although most of the presentations are related to tropical

islands, some method-oriented presentations were included that could be applied to these islands as well.

method for enterococci with correlative assessment for molecular markers for sewage contamination in selected environm

Evaluation of rapid QPCR method for enterococci with correlative assessment for molecular markers for sewage contamination in selected environmental water samples from Hawaii.

Basic Information

Title:	Evaluation of rapid QPCR method for enterococci with correlative assessment for molecular markers for sewage contamination in selected environmental water samples from Hawaii.
Project Number:	2012HI355B
Start Date:	3/1/2012
End Date:	2/28/2014
Funding Source:	104B
Congressional District:	Hawaii 1st
Research Category:	Water Quality
Focus Category:	Water Quality, Methods, Surface Water
Descriptors:	
Principal Investigators:	Marek Kirs

Publications

There are no publications.

Problem and Research Objectives

In a consent decree, USEPA agreed to address many criticisms of its current recreational water quality standards which were based on monitoring for two traditional fecal indicator bacterias (FIB) *Escherichia coli* and enterococci by 2012. However, USEPA was not able to properly evaluate the methods and application of alternative fecal indicators such as *Clostridium perfringens*, coliphages and Bacteroidales. As a result, USEPA has proposed to continue to use the same recreational water quality standards and have only added the use of a rapid molecular method (qPCR) for enterococci to be implemented in 2013. The proposed USEPA recreational water quality criteria have raised two potential problems.

The first problem was previously identified and will not be addressed by continuing to use the same recreational water quality standards. The current and future recreational water quality standards are based on the assumption that the source of FIB in recreational waters is sewage and correlated to risk levels for sewage borne infections among swimmers. This was determined by epidemiological studies when the source of contamination was known to be sewage. However, in epidemiological studies, when the source of FIB is environmental rather than sewage, the same risks associated with current recreational water quality standards would not apply. Previous studies have shown that tropical environments favor growth of FIB. This observation is the basis for higher concentrations and persistence of *E. coli* and enterococci in environmental sites (soil, sediments, sand, plants), especially in the state of Hawaii. An identified problem in the application for culture based or qPCR based method to monitor for *E. coli* and enterococci, is that these methods measure all sources of FIB and do not distinguish between sewage sources, animal sources, and environmental sources. The identified need to monitor environmental waters in the state of Hawaii, is to correlate the concentrations of enterococci by culturable and qPCR methods with selected Microbial Source Tracking (MST) assay which shows the presence and concentration of sewage contamination. In addition, the identified need is to assay the same water samples for culturable levels of *C. perfringens* as previous data have shown that monitoring for *C. perfringens* provides more reliable data for presence of sewage than monitoring for *E. coli* or enterococci.

The second identified problem was to establish and validate the reliability of the qPCR method when applied to environmental waters in Hawaii. In this regard, the Hawaii state Department of Health (HIDOH) as well as the City and County of Honolulu (CCH) recognized that they did not have the personnel to validate the qPCR method for enterococci. As a result, these two water quality monitoring agencies have concluded that they would require the assistance of Water Resources Research Center (WRRC) at the University of Hawaii to initially evaluate and establish a reliable/feasible qPCR method that can be applied to water samples obtained from the state of Hawaii. In addition to establishing a method to validate the reliability of the qPCR method, there are three other unknown environmental factors that can influence the qPCR results for enterococci from water samples obtained from a given area. The first environmental factor is related to the levels of inhibition in the water samples that are assayed by the qPCR method. There is evidence of more inhibition when water samples from tropical environments are assayed by the qPCR method. In a recent study conducted at the Boquerón Beach (Puerto Rico), 34% of the samples contained significant levels of PCR inhibitors. The second environmental factor relates to how environmental sources of enterococci, as compared to sewage sources, will react to the qPCR assay method. The third environmental factor relates to the expected ratio of dead to live enterococci populations in water samples obtained from

Hawaii. In summary, it was proposed that a trained molecular microbiologist from WRRC evaluate and establish a reliable qPCR method for enterococci when applied to water samples obtained from the state of Hawaii. It was also recommended that the laboratory personnel from the HDOH and CCH are trained in this method. As a result, the WRRC microbiologist will be in direct communication with the laboratory personnel from HDOH and CCH to share information and water samples for assays.

This project will provide an assessment of the rapid qPCR test for enterococci (USEPA Method 1611) in Hawaii and therefore prepare HDOH and CCH to the changes in the federal water quality criteria. We will work with HDOH and CCH to provide training as well as provide relevant information to their public outreach programs as requested. Results of the study will enable HDOH to identify if the USEPA's novel rapid test for enterococci is suitable in Hawaii and if the test should be incorporated into the State's water quality regulations.

Novel molecular tests will improve current microbial water quality monitoring programs and are needed for meaningful water management decisions in Hawaii. While the technology addressed in this project is applicable in all high priority areas identified by the Water Resources Research Institute Program, the project is centered on 'Water Quality' and has the following objectives:

1. Establish and evaluate performance of rapid qPCR test for enterococci (USEPA draft protocol A) in parallel with cultivation based assays in Hawaii.
2. Establish and evaluate qPCR based MST tools in Hawaii.

Methodology

Sample Collection

This research focuses on the island of Oahu. Extension of this research to other Hawaiian Islands has been discussed with the Clean Water Branch (DOH) and is dependent on the outcome of this project. Wastewater influent and effluent samples are currently being collected from the Sand Island Wastewater Treatment Plant (WWTP) as positive controls of human sewage and examined for signal decay. Surface water samples are being collected in locations relevant to the CCH and DOH needs. In this regard we just completed sampling from a sewage spill near Keehi Lagoon, another two sets of samples will be collected after the spill is contained. Ala Wai channel and Palolo Stream are also being sampled. Another ten coastal sites have been identified by DOH for this project and are also being sampled at monthly intervals. A minimum of 70 samples will be analyzed.

Laboratory Analysis

Each water sample is being analyzed by culture based methods for enterococci, *E. coli*, and *C. perfringens* (spores and vegetative cells). Molecular tests are also being used to identify concentrations of enterococci (USEPA method 1611 [USEPA 2012]) and *C. perfringens* (Dumoncaux et al., 2006). Another set of molecular tests, targeting human *Bacteroides* and human polyomavirus groups, are being used to identify human contamination components. We are using standard USEPA approved protocols when available and published protocols for molecular source tracking markers.

Inhibition, positive (standards), extraction blanks and negative controls are being used. We use absolute standard based techniques to quantify molecular signals, except for the calibrator

based ($\Delta\Delta C_i$) method which is used for quantification of enterococci according to the USEPA guidelines (USEPA, 2012). Genomic standards were extracted and quantified from cultured clonal isolates (*Enterococcus faecalis* ATCC29212, *Clostridium perfringens* NCTC8798, *Bacteroides thetaiomicron* ATCC29741) obtained from the American Type and culture collection (ATCC) or in the form of bioball from the bioMérieux Inc. DNA concentration was determined on QBit 2.0 luminometer (Life Sciences) using factory provided standards. Target gene copy numbers were estimated using copy number calculator at the SciencePrimer (<http://www.scienceprimer.com/copy-number-calculator-for-realtime-pcr>). Human polyomavirus standard was purified and quantified from the cloned synthesized target containing required primer and probe sites at appropriate intervals. All plasmid based standards were linearized using a restriction enzyme (ApaI) and cleaned before use.

Validation of Source Tracking Markers

Human specific source tracking markers (human *Bacteroides*, and polyomavirus) are being validated for the specificity and sensitivity using animal and human fecal samples. Human, rodents, cats and wild pigs are being targeted. Also, concentrations of enterococci in the fecal samples is being determined using cultivation based techniques.

Permits to import micro-organisms (controls), as well as IRB permits to work with human subjects were obtained for this project from January–March 2013.

Principal Findings and Significance

This project is ongoing. The following is a brief summary of our progress.

Sample Collection

Two sets of influent and effluent, two sets of surface water (9 sites each), and one set of coastal samples (10 sites) have been collected. Sample collection is ongoing.

Laboratory Analysis

While cultivation based techniques can be applied routinely without much of an effort for setup, molecular testing requires time to setup and thorough validation of the test is needed before environmental samples can be reliably analyzed. Obtaining the required permits and ordering the components have delayed the project.

QPCR protocols for enterococci, *C. perfringens*, human *Bacteroides* and human polyomavirus have been setup and validated using positive controls. Cell and/or genome based standards demonstrate good efficiency (95%–104%), indicating near perfect doubling of target material in all PCR reactions (Figure 1). The standards were linear over six logs of dilutions and down to 40–50 copies of target per PCR reaction could be reliably quantified. Lower concentrations have not been tested, but in theory up to one copy per reaction should be detectable. We have encountered no problems with extraction blanks or negative controls.

Current ‘surface water’ data, which does not include molecular components, is included in Table 1. It demonstrates high concentrations of *E.coli* and enterococci in freshwater samples, likely originating from environmental pools as *C. perfringens* concentrations were not elevated. The Nimitz culvert site was known to leak sewage from a broken sewer main as indicated by a recent dye tracer study conducted by DOH. While the high enterococci concentrations were

detected in the site, *C. perfringens* spore concentrations were not elevated. This requires further investigation as *C. perfringens* is known to be a good indicator of sewage contamination and the sewer main is still scheduled for repairs. It would be premature to speculate solely based on those two sets of samples, and molecular data analysis has not been completed. The study is ongoing.

Molecular tests on environmental samples will be completed once all samples have been received due to the costs associated with multiple run setups, and to minimize run to run variations.

Validation of Source Tracking Markers

We have finished collecting cat (10) and rodent samples (10 rat, 10 mongoose), and are currently collecting samples from human volunteers and pigs. All collected samples have been analyzed for enterococci concentration, and extraction of DNA has been completed. DNA is stored for molecular tests, which will be completed once all samples have been received due to the costs associated with multiple run setups, and to minimize run to run variations. Concentrations of enterococci varied in rat fecal samples from 555–>23,000, and in cat fecal samples from 34080–>96,000 MPN per 1 g. This variation could be attributed to variability between the individuals, although some variation is likely due to the sample age.

Within the framework of this project, we have strengthened our partnership with the DOH (Clean Water Branch) and CCH (Laboratory Branch, Department of Environmental Services). Opportunities to extend this study to other islands is being discussed with HDOH. Also, methods and tools validated in this study could be used and transferred to our partner laboratories (DOH, CCH) as needed.

Dr. Mayee Wong, a postdoctoral researcher, was hired in January 2013 partially from the funds of this project. She has been trained in all the methods used in this study and will continue to support this project.

We envision that this study can support our NOAA and Sea Grant funding proposals as well as gain further interest and support from DOH and CCH once this project is completed.

Publication Cited in Synopsis

Dumonceaux, T.J., J.E. Hill, S.A. Briggs, K.K. Amoako, S.M. Hemmingsen, and A.G. Van Kessel, 2006, "Enumeration of specific bacterial populations in complex intestinal communities using quantitative PCR based on the chaperonin-60 target," *Journal of Microbiological Methods*, 64, 46–62.

USEPA, 2012, Method 1611: Enterococci in Water by TaqMan® Quantitative Polymerase Chain Reaction (qPCR) Assay, Office of Water, Washington DC, Washington, p. 37.

Figure 1. Amplification curves of serially diluted standards for molecular enterococcus (A and B) and human polyomavirus (C and D) test. Insert A also indicates uniform (inhibition free) amplification of inhibition controls (Salmon testes DNA assay).

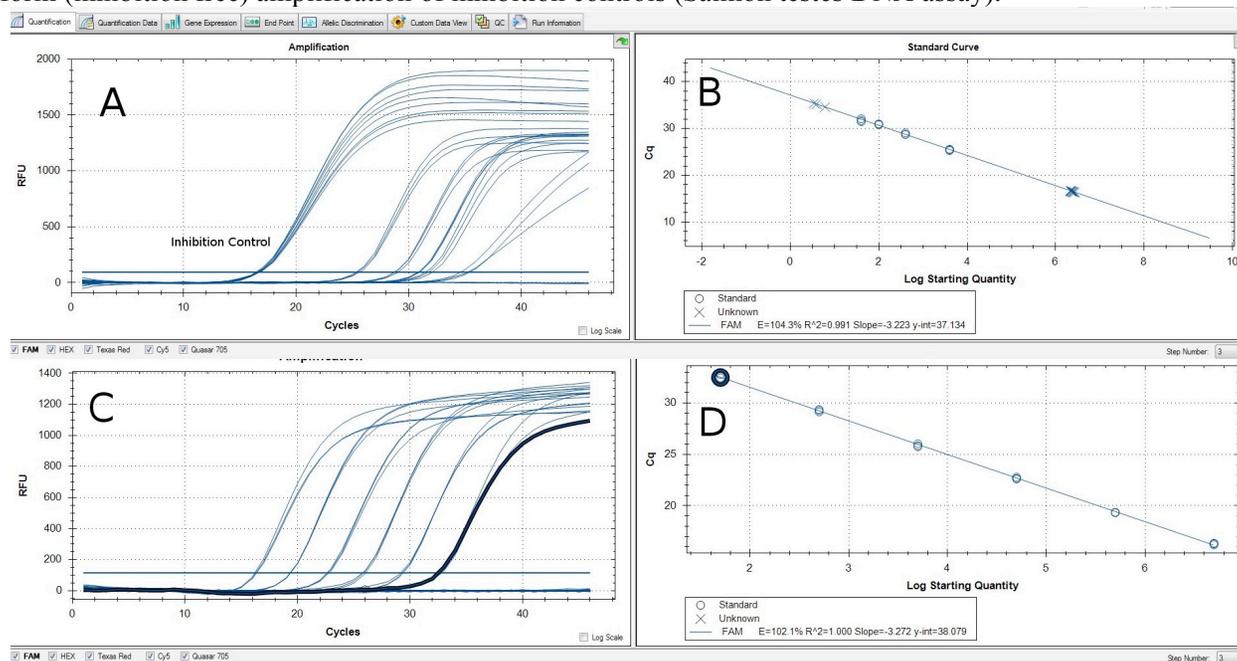


Table 1. Indicator bacteria concentrations in two sets of surface water samples.

	Salinity (ppt)	<i>E. coli</i> (MPN/100 ml)	Enterococci (MPN/100 ml)	<i>C. perfringens</i> (MPN/100 ml)
Nimitz Road Bridge	24.1-26.6	390-1935	121-613	<1-7
Nimitz Culvert	17.8-21.4	3448-6867	341-1467	<1-15
DAV Bridge	24.4-27.3	512-805	98-355	14
DAV	25.0-28.5	309-420	110-490	8 - 27
Ala Moana Beach	33.0-33.5	41-97	<10-10	<1
Ala Wai Mouth	19.9-28.6	173-6131	97-201	1-4
Ala Wai Canoe Club	7.8-18.8	446-5764	52-691	2-26
Lower Palolo Stream	<0.1	4884-5475	602-12997	<1-49
Upper Palolo Stream	<0.1	4611-3873	697-6488	<1

Promoting Water Sustainability Literacy

Basic Information

Title:	Promoting Water Sustainability Literacy
Project Number:	2012HI356B
Start Date:	3/1/2012
End Date:	2/28/2014
Funding Source:	104B
Congressional District:	Hawaii 1st
Research Category:	Not Applicable
Focus Category:	Education, Water Use, Conservation
Descriptors:	
Principal Investigators:	John Cusick, David C. Penn

Publications

There are no publications.

Problem and Research Objectives

Hawaii faces immediate challenges in meeting water demands and implementing wastewater and stormwater reuse programs. Despite our location in the middle of the Pacific Ocean, our water resources are very limited and Hawaii's ecosystems and environments are increasingly threatened by drought and the impacts of environmental degradation and climate change. The purpose of this water literacy project is to address complex human-environment interactions associated with water resource management, as mediated by the diverse cultural, political, and economic conditions in the State of Hawaii. In particular, we focus on educating the University of Hawaii at Manoa (UHM) campus community to raise literacy about water-related issues at the campus, neighborhood, watershed, and island scales.

Methodology

This project provides opportunities for students to learn about water resource management challenges through the process of planning, organizing, and attending campus events that focus on promoting water sustainability literacy. Each semester, we recruit undergraduate students from two UHM interdisciplinary programs—Environmental Studies and Honors—to collaborate on project activities with faculty and staff of the UHM Environmental Center and the UHM Manoa Sustainability Council. These activities—including research seminars, panel discussions, and showcases of student work—provide opportunities for individuals from across disciplines to engage with fellow members of the campus community and further the objectives of campus stewardship as identified in the UHM Strategic Plan.

Although there are numerous events to attend on campus in any given semester, the water forums focus the attention of the campus community on a specific aspect of sustainability literacy. Students address water resource management challenges by developing, articulating, and disseminating knowledge through collaboration with faculty, fellow students, businesses, and government agencies. Student involvement includes event planning and promotion; independent research, and the preparation of conference posters and exhibit materials in collaboration with Water Resources Research Center (WRRC) faculty and affiliates. The project budget also includes funding for the completion of rainfall catchment demonstration structures at WRRC's Krauss Annex facility.

Principal Findings and Significance

Our review of event content and participation indicates that the goal of sustainability literacy, in general, is a welcome addition to the campus conversation. Among the principal findings is a need to improve coordination among members of the campus community who are already engaged with the topics of environmental education, sustainability literacy, and water resource management. Nonetheless, attendance at water sustainability literacy events totaled over two hundred people, averaging about 20 people per event.

The events were planned and organized by several dozen students and faculty. One of the major considerations in the planning process was when and where to stage these events in order to maximize future learning outcomes and increase the audience for water sustainability literacy.

Of particular significance is the timeliness of the topics and events in relation to the current development of on-campus and off-campus academic programs, course proposals, and research and service projects. The water literacy events conducted during this project contribute to the achievement of sustainability education and awareness training that is promoted in the current campus strategic plan (University of Hawaii at Manoa, 2011). The topics covered are linked with specific objectives established in the UHM Chancellor's "Sustainability Policy Statement" such as maximizing water conservation, water efficiency, and best management practices for stormwater storage, recharge, and reuse (University of Hawaii at Manoa, 2012).

In Fall 2012, the water sustainability literacy project culminated in a Sustainability Open House that showcased seven off-campus student internships and fifteen on-campus projects initiated by students (December 5–6, 2012). The UHM Chancellor endorsed one of these projects in a letter of support for a student led proposal to the U.S. Environmental Protection Agency (Cusick, 2012). Student learning outcomes also included expanded professional networks and the development of research and communication skills of interest to employers.

The sustainability literacy events conducted during this project provided a focus for student preparation and presentation of web-based resources, including YouTube videos, PowerPoint slides, conference posters, and project base maps that can be used for future reference by the campus community. These work products will be further developed and archived during the coming project year, led by students in the Fall 2013 Environmental Studies Practicum course. The original design of the rainfall catchment demonstration structures will be augmented through the additional financial support generated from a successful, student-driven proposal to the UHM Student Athletic Fee Committee (Krauss Neighborhood Makeover Project).

Publications Cited in Synopsis

Cusick, J., 2012, Modified rain garden to increase sustainability literacy on the University of Hawaii at Manoa Campus, proposal to the *10th Annual P3 Awards: A National Student Design Competition for Sustainability Focusing on People, Prosperity, and the Planet*, U.S. Environmental Protection Agency, Extramural Research.

Manoa Sustainability Council (MSC), 2012, Achieving our destiny: 2011–2015 strategic plan initiative 4 (MSC revision), *Sustainability at UH Manoa*, <http://manoa.hawaii.edu/sustainability/2012/09/achieving-our-destiny-2011%E2%80%902015-strategic-plan-initiative-4>.

University of Hawaii at Manoa, 2011, Achieving our destiny—the University of Hawai'i at Manoa 2011–2015 strategic plan, <http://manoa.hawaii.edu/vision/pdf/achieving-our-destiny.pdf>.

University of Hawaii at Manoa, 2012, UHM sustainability policy statement (October 2012), *Sustainability at UH Manoa*, <http://manoa.hawaii.edu/sustainability/2012/10/uhm-sustainability-policy-statement-october-2012>.

Satisfying Growing Water Demand Through Integrated Groundwater and Watershed Management

Basic Information

Title:	Satisfying Growing Water Demand Through Integrated Groundwater and Watershed Management
Project Number:	2012HI361B
Start Date:	3/1/2012
End Date:	2/28/2013
Funding Source:	104B
Congressional District:	Hawaii 1st
Research Category:	Social Sciences
Focus Category:	Economics, Management and Planning, None
Descriptors:	
Principal Investigators:	Kimberly Burnett

Publication

1. Burnett, Kimberly and Christopher Wada, 2013, Optimal groundwater management when recharge is declining: A method for valuing the recharge benefits of watershed conservation, Environmental Economics and Policy Studies. (in preparation)

FINAL REPORT

**Satisfying Growing Water Demand Through Integrated
Groundwater and Watershed Management**

May 2013

Kimberly Burnett

Project Number: 2012HI361B

Water Resources Research Center
University of Hawaii at Manoa
Honolulu, Hawaii

Problem and Research Objectives

Water tables and stream flow in Hawai‘i have been declining over the past century in the face of increasing demand (Bassiouni and Oki, 2012), and demand will continue to increase as per capita incomes rise and the population grows. Climate change can exacerbate the problem through changes in precipitation patterns and quantities, evapotranspiration, and land cover, all of which directly or indirectly affect the amount of water that ultimately infiltrates back into the ground. If the climate becomes less favorable to groundwater recharge as preliminary projections suggest, the importance of efficient management of both supply and demand options will be paramount.

Sustaining water availability at current prices in the face of growing demand and declining resources is highly unlikely. Therefore, our research aims to develop a management framework with the objective of conserving water resources in a manner that maximizes water users’ benefits over time, given projected effects of a continuously changing climate. Specifically, we consider long-term planning for efficient extraction of groundwater and timing for the development of groundwater alternatives such as desalinated water when recharge is declining. Recharge-enhancing watershed conservation would further help to moderate scarcity, resulting from the dual effect of increasing demand and declining recharge, and thus positively affect social welfare. By comparing welfare estimates under different assumptions about recharge, including different rates of decline and maintenance, we are able to indirectly estimate a lower bound for ecosystem benefits associated with hypothetical conservation projects.

Methodology

The methodology consists of three main components or modules: climate, groundwater, and management. Results from currently available analyses of climate, land cover, and watershed hydrology are used to construct three basic recharge scenarios. Although considerable assumptions are necessary to do so, the resulting framework is operational and useful for illustrating the tradeoffs involved in implementing different management tools. The groundwater module is based on a single-cell aquifer model, and the management module ties all of the components together in a socioeconomic dynamic optimization framework.

Climate module

Although historical trends of air temperature and precipitation in Hawai‘i have been extensively documented (Chu and Chen, 2005; Chu et al., 2010; Giambelluca et al., 2008), few studies have attempted to simulate future climate change in Hawai‘i at a regional scale. Timm and Diaz (2009) apply statistical downscaling to rainfall of the Hawaiian Islands under the assumption that GCMs reasonably simulate large-scale atmospheric circulation patterns. Based on a six-model ensemble selected from the models presented in the Intergovernmental Panel for Climate Change Fourth Assessment Report (Christensen et al., 2007), their results suggest that the most likely scenario for Hawai‘i is a 5–10% reduction in precipitation during the wet-season (November–April) and a 5% increase during the dry season (May–October) by the end of the twenty-first century. Given that approximately 70% of normal precipitation falls during the wet season (Safeeq and Fares, 2012), the net effect is a decline in annual precipitation and hence groundwater recharge.

Previous studies have provided recharge estimates ranging from 19% to 43% of gross annual rainfall across various sites throughout Hawai‘i. However, changes in precipitation may be accompanied by changes in other climate variables, which affect water balance in the watershed. Safeeq and Fares (2012) use data from the Mākaha watershed to assess the sensitivity of streamflow and evapotranspiration (ET) to a variety of future climate change models, including two scenarios based on precipitations changes projected by Timm and Diaz (2009). Assuming that ET, streamflow, and recharge comprise 56%, 11% and 33% of rainfall respectively, we construct a conservative scenario, corresponding to a 1.9% decline in precipitation and 3.7% decline in recharge, and a baseline scenario, corresponding to a 5.3% decline in precipitation and 8.5% decline in recharge by the end of the twenty-first century.

Although the estimates are based on the Mākaha watershed, we extend the results to the neighboring Ko‘olau watershed, which recharges the large and heavily used Pearl Harbor aquifer. The responsiveness of each watershed to climate change is not realistically identical. Nevertheless, we aim to illustrate how the present value of a groundwater resource can vary even for a seemingly small change in precipitation over the next century, and how that result can be used to value watershed conservation programs that aim to slow or halt the expected decline in groundwater recharge. We construct a time-dependent groundwater recharge function as follows:

$$R(t) = R_0(1 - \delta t / \tau) \quad (1)$$

where R_0 is the current value of recharge, δ is the projected percentage reduction in precipitation relative to R_0 for year 2100, and τ is the total number of periods of expected recharge reduction, in this case, 87 years. The result is a linear reduction in annual recharge from R_0 to $R(\tau)$.

Groundwater module

The recharge estimates serve as inputs to a groundwater framework based on Liu’s (2006) Robust Analytical Model 2, which characterizes solute-transport within a vertical cross-section of a coastal aquifer. The governing or state equation, which describes the evolution of groundwater stock over time, is

$$\gamma \dot{h}_t = R(t) - l(h_t) - q_t \quad (2)$$

where γ converts head level to stored groundwater volume, h_t is the head level at time t , $R(t)$ is groundwater recharge for period t , q_t is the quantity of groundwater extracted at time t , and l is a leakage function, parameterized for Pearl Harbor by Krulce et al. (1997). Taking into account average well-depth, upconing, and the desirable source-water salinity in Hawai‘i (2% of seawater salinity), management decisions are constrained to prevent the head level for Pearl Harbor aquifer from falling below 15.125 feet, the minimum allowable head level (h_{min}):

$$h_t \geq h_{min} \quad (3)$$

Economic module

The benefit of groundwater use is calculated as consumer surplus, or the area under the inverse demand curve. Demand is modeled as a constant elasticity function:

$$D(p_t, t) = \alpha e^{gt} p_t^\eta \quad (4)$$

where η is the elasticity of demand, g is the rate of demand growth, and α is a coefficient selected to normalize equation (4) to Honolulu Board of Water Supply (HBWS) data for the average

retail price of water and extraction from the year 2009. Non-HBWS pumpage is taken as exogenous and is assumed to grow at rate g .

The marginal cost of extracting groundwater is specified as a linear function of the distance water must be lifted from the aquifer to the surface:

$$c(h_t) = \beta(e - h_t) \quad (5)$$

The coefficient (β) is chosen such that the cost calculated using equation (5) together with data for average well elevation (e) and the initial head level (h_0) matches the volume-weighted average of unit extraction costs for all primary wells in the initial period. The initial head level of 17.1 feet is estimated by taking the average of water levels measured at six monitoring wells (Moanalua, Halawa, Kalauao, Pearl City, Waipahu, and Hoaeae-Kunia) over the period 2009–2012. The unit cost of distribution (c_d) is calculated as the difference between the retail price and the unit extraction cost in 2012 dollars. The inflation-adjusted unit cost of desalinating seawater using reverse osmosis (c_b) includes amortized capital costs.

The optimization problem for a forward-looking planner is to choose extraction and desalination in every period, given a discount rate (r) to maximize the present value of net benefits to society, i.e.

$$\text{Max}_{q_t, b_t} \int_{t=0}^{\infty} e^{-rt} \left\{ \int_{x=0}^{q_t + b_t} D^{-1}(x, t) dx - [c(h_t) + c_d] q_t - [c_b + c_d] b_t \right\} dt \quad (6)$$

subject to equations (2), (3), and non-negativity constraints on the control variables. We repeat the optimization procedure for each of the recharge scenarios, including maintenance of the status quo level. Table 1 provides descriptions of the various parameters used to characterize equations associated with each of the three modules.

Table 1. Parameter descriptions, units, and values.

Parameter	Description [units]	Value
R_0	Initial recharge [mgd]	220
δ	Projected change in recharge by 2100 [-]	-0.037
τ	Length of climate projection [years]	87
h_0	Initial head level [feet]	17.1
h_{min}	Minimum head level [feet]	15.125
α	Demand coefficient [mgd/\$]	107.4
g	Rate of demand growth [-]	0.01
η	Elasticity of demand for water [-]	-0.25
β	Extraction cost coefficient [\$/foot/tg]	0.00137
e	Average well elevation [feet]	272
c_d	Unit distribution cost [\$/tg]	3.39
c_b	Unit cost of desalination [\$/tg]	8.46
r	Discount rate [-]	0.03

Principal Findings and Significance

In the baseline scenario, desalination is not implemented until year 77. The net present value (NPV) of the resource is \$7.71 billion, and the benefit of maintaining recharge at the current level, calculated as the difference in NPV when recharge is maintained and when it is declining, is \$170.5 million. Watershed conservation that maintains recharge at the current level is only warranted if the NPV costs do not exceed \$170.5 million. Thus, even when groundwater is abundant and recharge is projected to decline moderately, the value of watershed conservation can be substantial.

Table 2. Sensitivity analysis.

Scenario	δ [recharge decline]	T [years until desalination]	h(T) [feet]	NPV [millions]	Benefits of conservation [millions]	PV gain relative to no conservation [percent]
Baseline	0%	81	hmin	\$7,885.7	-	-
	3.7%	77	hmin	\$7,721.8	\$163.9	2.12%
	8.5%	73	hmin	\$7,538.0	\$347.7	4.61%
High demand growth ($g=0.03$)	0%	34	hmin	\$6,481.9	-	-
	3.7%	34	hmin	\$6,450.8	\$31.1	0.48%
	8.5%	33	hmin	\$6,288.8	\$193.1	3.07%
Elastic demand ($\eta=-0.5$)	0%	87	hmin	\$6,529.5	-	-
	3.7%	87	hmin	\$6,511.2	\$18.3	0.28%
	8.5%	87	hmin	\$6,476.3	\$53.2	0.82%
Low discount rate ($r=0.01$)	0%	83	hmin	\$15,804.7	-	-
	3.7%	79	hmin	\$15,053.9	\$750.8	4.99%
	8.5%	75	hmin	\$14,272.7	\$1,532.0	10.73%
High extraction cost ($\beta=0.00274$)	0%	82	hmin	\$7,530.9	-	-
	3.7%	78	hmin	\$7,377.9	\$153.0	2.07%
	8.5%	74	hmin	\$7,206.0	\$324.9	4.51%

To explore the model's sensitivity to assumed parameter values, we run simulations with different values of g , η , β , and r , for high and low recharge scenarios. When recharge decline is projected to be relatively small and groundwater is fairly abundant, changes to some parameters do not substantially affect the optimal management strategy. For example, lowering the discount rate to 1% or doubling the extraction cost parameter does not have much effect on the timing of desalination. Increasing g to 3% brings desalination much closer to the present (year 34),

however, inasmuch as groundwater must be drawn down faster to meet growing demand. Doubling the elasticity of demand has the opposite effect because consumers are more responsive to price.

When recharge is expected to decline to 8.5% of its current level by the end of the century, the qualitative effects (i.e., the direction of the changes) on PV benefits of perturbations to various parameter values remain intact. Furthermore, the value of maintaining recharge is higher if recharge is expected to decline by a larger amount absent watershed management, as evidenced by the last two columns in Table 2. For each scenario, the PV benefit of maintaining recharge is at least twice as large for a recharge decline of 8.5% as it is for a decline of 3.7%. The difference in benefit in percentage terms is especially large for the high demand growth scenario; when water is already scarce owing to increased demand, maintaining recharge is especially valuable.

Our scenario-based analysis focuses on a single hydrological basin. Future research could be aimed at extending the framework in a variety of directions. Increasing the management boundaries to encompass the entire island of O‘ahu would require a systems approach, including the characterization of natural flow between the various groundwater sectors, distribution or allocation of water between sectors, and the sequencing of all available water sources over space and time. The framework would also be improved by explicitly incorporating stochasticity, with the objective of maximizing the expected present value of the resource, given projected distributions of uncertain climate variables. Lastly, including investment in watershed conservation as a management instrument, i.e., not taking a project-based approach, would improve the utility of the model for decision makers; maintaining recharge at current levels is likely to be costly and less conservation may yield higher net benefits.

References

- Bassiouni, M. and D.S. Oki, 2012, Trends and shifts in streamflow in Hawai‘i, 1913–2008, *Hydrological Processes*, doi: 10.1002/hyp.9298.
- Christensen, J.H., B. Hewitson, A. Busuioc, A. Chen, X. Gao, I. Held, R. Jones, R.K. Kolli, W.-T. Kwon, R. Laprise, V. Magaña Rueda, L. Mearns, C.G. Menéndez, J. Räisänen, A. Rinke, A. Sarr and P. Whetton, 2007, Regional Climate Projections, in *Climate Change 2007: The Physical Science Basis, Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*, edited by S. Solomon et al., Cambridge University Press, Cambridge, United Kingdom and New York.
- Chu, P.S. and H. Chen, 2005, Interannual and interdecadal rainfall variations in the Hawaiian Islands in a warming climate, *Journal of Climate*, 18, 4796–4813.
- Chu, P.S., Y.R. Chen and T. Schroeder, 2010, Changes in precipitation extremes in the Hawaiian Islands in a warming climate, *Journal of Climate*, 23, 4881–4900.
- Giambelluca, T.W., H.F. Diaz and M.S.A. Luke, 2008, Secular temperature changes in Hawai‘i, *Geophysical Research Letters*, 35, L12702.
- Krulce, D.L., J.A. Roumasset and T. Wilson, 1997, Optimal management of a renewable and replaceable resource: The case of coastal groundwater, *American Journal of Agricultural Economics*, 79, 1218–28.

- Liu, C.C.K., 2006, Analytical Groundwater Flow and Transport Modeling for the Estimation of the Sustainable Yield of Pearl Harbor Aquifer, Project Report PR-2006-06, Water Resources Research Center, Honolulu.
- Safeeq, M. and A. Fares, 2012, Hydrologic response of a Hawaiian watershed to future climate change scenarios, *Hydrological Processes*, 26, 2745–2764.
- Timm, O. and H.F. Diaz, 2009, Synoptic-statistical approach to regional downscaling of IPCC twenty-first-century climate projections: Seasonal rainfall over the Hawaiian Islands, *Journal of Climate*, 22, 4261–4280.

Fate and Transport of Pharmaceutically Active Compounds in Simulated Bank Filtration System

Basic Information

Title:	Fate and Transport of Pharmaceutically Active Compounds in Simulated Bank Filtration System
Project Number:	2012HI362B
Start Date:	3/1/2012
End Date:	2/28/2014
Funding Source:	104B
Congressional District:	Hawaii 1st
Research Category:	Ground-water Flow and Transport
Focus Category:	Water Supply, Water Quality, Geochemical Processes
Descriptors:	
Principal Investigators:	Chittaranjan Ray, Matteo D'Alessio

Publications

1. D'Alessio, M., and C. Ray, 2013, "Environmental fate and transport of selected pharmaceutically active compounds (PhACs) during riverbank filtration," presented at 2013 World Environmental & Water Resources Congress, Cincinnati, Ohio, May 19–23.
2. D'Alessio, M., and C. Ray, 2013, "Environmental fate of selected PhACs during RBF." (in preparation for submission to Water Research, July 2013)
3. D'Alessio, M., and C. Ray, 2013, "Impact of clogged and unclogged conditions at a model RBF site on the dynamic of redox conditions and on the fate and transport of selected PhACs." (in preparation for submission, Fall 2013)

Problem and Research Objectives

Riverbank filtration (RBF) represents a natural filtration technique that has been used to provide drinking water to communities for more than a century in Europe and half a century in the United States. RBF uses the riverbed and the underlying aquifer as natural filters to remove pollutants present in the surface water (Ray et al., 2002). As the infiltrating water moves through the porous media, dissolved contaminants as well as pathogens are removed (Ray et al., 2002). RBF is also able to moderate temperature fluctuations observed in surface waters as well as concentration peaks resulting from accidental spills (Ray et al., 2002). Straining, colloidal filtration, sorption and microbial degradation contribute to the removal and attenuation of these contaminants (Ray and Shamrukh, 2011).

Appearance of pharmaceutically active compounds (PhACs) are found in surface water sources at an increasing rate and the mechanism of removal during RBF has become a concern (Hiscock, and Grischek, 2002). Several PhACs from different prescription drugs have been found at concentrations up to the $\mu\text{g/L}$ -level in sewage influent and effluent samples and also in several surface waters located downstream from municipal sewage treatment plants (Caliman and Gavrilescu, 2009).

The objectives of this study were to evaluate the potential impact of a) variable redox conditions; b) variable organic matter content of the source water; c) seasonal variability of temperature; d) presence versus absence of riverbed sediments; and e) changes in solution ionic strength coupled with increased solution velocity on the fate, transport, and removal of several PhACs during their passage in a model RBF system.

Methodology

PhACs were selected according to their pharmaceutical class, the occurrence in the environment and public interest, their toxicity, the environmental fate, the behavior under different redox conditions, and the availability of analytical standards and adequate instrumentations. The fate and transport of these selected PhACs was investigated using short columns (Figure 1) and a two-side-by-side rectangular slab-like column (Figure 2). Short columns were used to evaluate the impact of temperature, organic content, redox conditions and bacteria on the fate and transport of selected PhACs, and to reduce the possible scenarios that are simulated in the slab. On the other hand, the slab was used to evaluate the impact of clogging on the dynamic of redox conditions as well as the removal of selected PhACs.



Figure 1. Four columns used for the short-column simulations.

Four flow-through stainless steel columns (4.75-cm internal diameter and 14.50-cm height) were simultaneously used in this study to observe the transport of PhACs through the columns (Figure 2). Light-excluding stainless steel columns were selected in order to minimize the possible effect of photo-degradation of the selected PhACs as well as the potential adsorption of these compounds on the internal wall of the column. Silica sand (#16 to #30 sieve) was acclimated in the presence of the long column (Figure 3) prior to being used as packing material for the short columns. Experiments were performed under aerobic and anaerobic conditions. In order to achieve aerobic conditions, air was continuously bubbled into the reservoir, while anaerobic conditions were obtained by keeping the reservoir closed and by purging the reservoir with nitrogen. The seasonality impact was evaluated by performing the different experiments in the laboratory (20°C) and in a refrigerator room (6°C). The effect of different levels of organic carbon on the behavior of selected PhACs was evaluated using Lake Wilson (low content) and Lake Wilson with different levels of humic acid to increase the level of organics. Sterile conditions, achieved by autoclaving silica sand 3 times at 120°C for 30 min, and filtering water collected from Lake Wilson through 0.2 µm filter and autoclaving at 120°C for 30 min, were used as abiotic control.

The 140 x 60 x 10 cm slab, made of transparent plexiglas, was covered with a thick black cardboard in order to prevent the possible microbial growth due to the light penetration through the plexiglas. The top of the slab was covered in order to reduce possible evaporation and the exposure to light. The slab was dry-packed with small increments of silica sand (#14 to #16 sieve, at the bottom, and #16 to #30 sieve) and manually compacted during this process. An overflow located 4 cm above the top layer of sand was used to maintain a constant water level above the sand. Two automatic balances, connected to a CR10X Campbell Scientific datalogger (USA), were used in order to have an in-line monitoring of the outflow. The experimental slab was equipped with oxygen and redox probes for online measurements, tensiometers to monitor pressure for clogged and unclogged conditions, and sampling ports to determine the evolution of the environmental conditions in the slab and to monitor the removal of PhACs at different depths. The number of probes and sampling ports was higher in the top section of the slab to

account for the removal occurring just beneath the biological layer (*Schmutzdeche*) formed on the top surface of the sand/soil. Offline samples were analyzed in terms of TOC, DOC, PhACs, and SUVA (254). To simulate clogged and unclogged conditions, a thin layer of fine sediments collected from Manoa Stream was added above the top portion of the sand on one side of the slab. This would enhance the development of a clogged layer at the interface of the sand/sediments.



Figure 2. Slab set-up with redox probes, oxygen probes, sampling ports and tensiometers.



Figure 3. One set of two long columns adopted for the long-column re-circulated simulations.

Principal Findings and Significance

The selected PhACs investigated during this study can be divided into:

1. PhACs persistent under different environmental conditions
2. PhACs removed only in presence of specific conditions

Sterile conditions were achieved and maintained for approximately 40 days during the study.

PhACs Persistent Under Different Environmental Conditions

Carbamazepine and gemfibrozil were persistent under aerobic and anaerobic conditions as well as during summer and winter. Limited removal (<20%) was observed in the presence of the different environmental simulated conditions. Figure 4 shows the behavior of carbamazepine in the presence of sterile and non sterile conditions, summer and winter, aerobic and anaerobic.

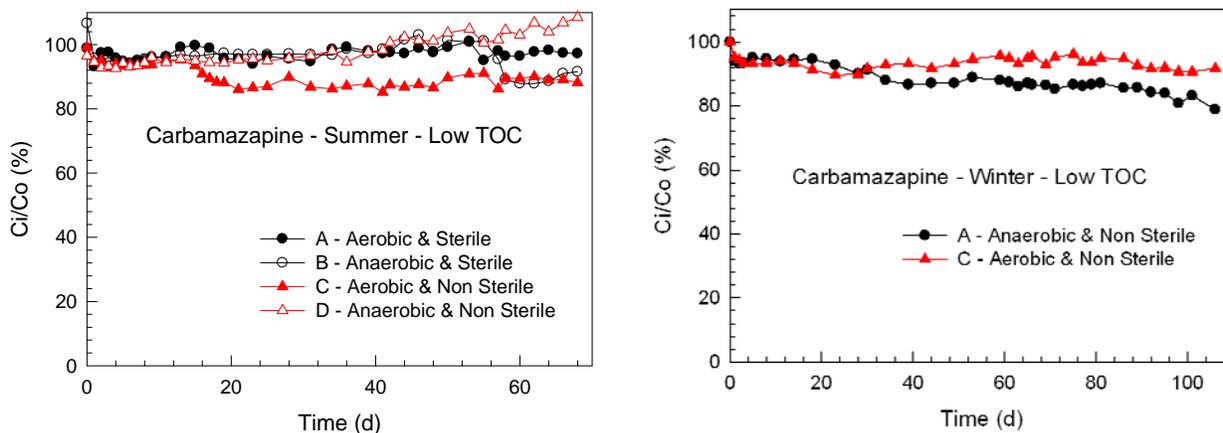


Figure 4. Impact of redox conditions and temperature on fate and transport of carbamazepine.

PhACs Impacted by Temperature and Redox Conditions

Results from this study suggest that the environmental behavior of phenazone and caffeine was highly impacted by redox conditions and seasonality at the bank filtration site. Under anaerobic conditions, both chemicals were persistent and low/limited removal (<15%) was observed (Figures 5–6). While under aerobic conditions, both chemicals were completely removed. Caffeine and phenazone were completely removed within 20 days during the summer, while only phenazone was removed during the winter. Phenazone required an acclimation period of 15 and 65 days during summer and winter, respectively. In the presence of cold temperature, the removal rate of phenazone was also slower compared to the removal rate observed during the summer.

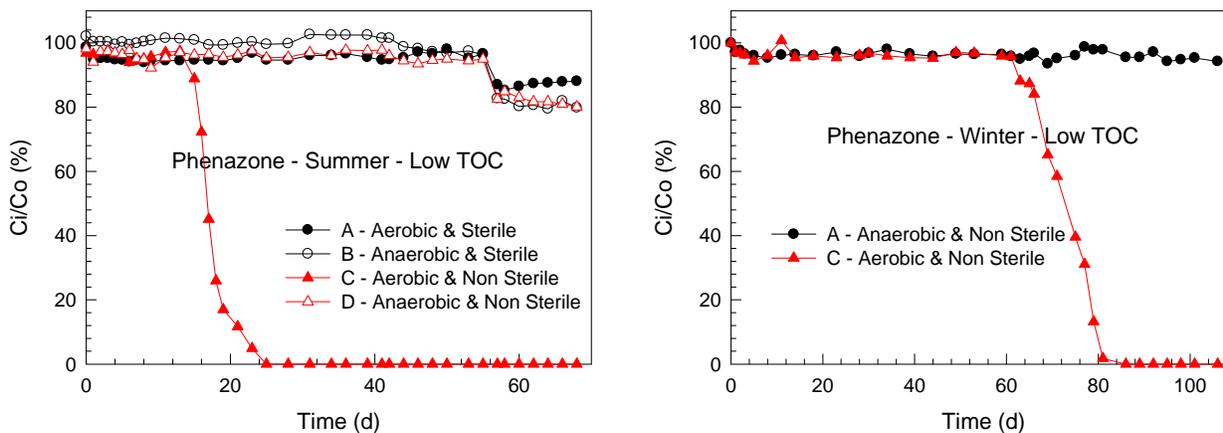


Figure 5. Impact of redox conditions and temperature on fate and transport of phenazone.

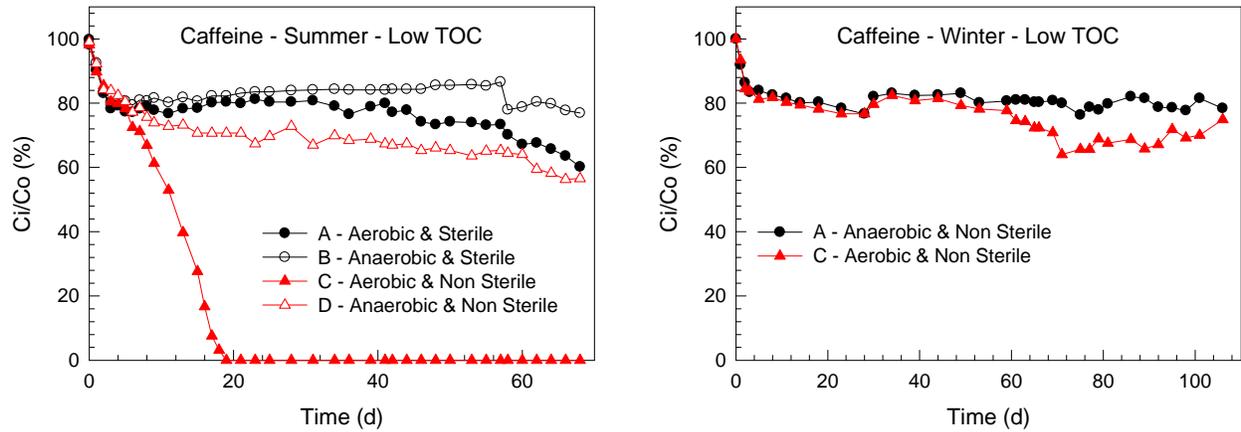


Figure 6. Impact of redox conditions and temperature on fate and transport of caffeine.

The behavior of two estrogens E1 and E2, was also impacted by temperature and redox conditions (Figures 7–8). Faster and more pronounced removal of both estrogens was observed in the presence of aerobic conditions. E1 and E2 were then completely removed within 22 days under aerobic conditions. Slower removal of E2 was observed under anaerobic conditions, while limited removal of E1 (<10%) was also observed. However, slower removal of E1 and E2 was observed under aerobic and anaerobic conditions during the winter. In the presence of sterile conditions, limited/no removal of both estrogens was observed (Figures 7–8).

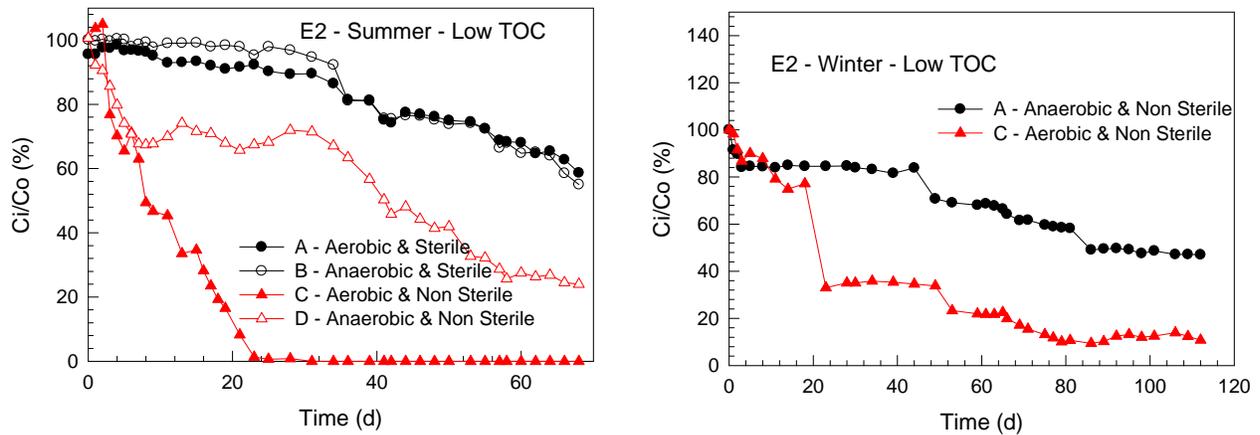


Figure 7. Impact of redox conditions and temperature on fate and transport of 17-β estradiol (E2).

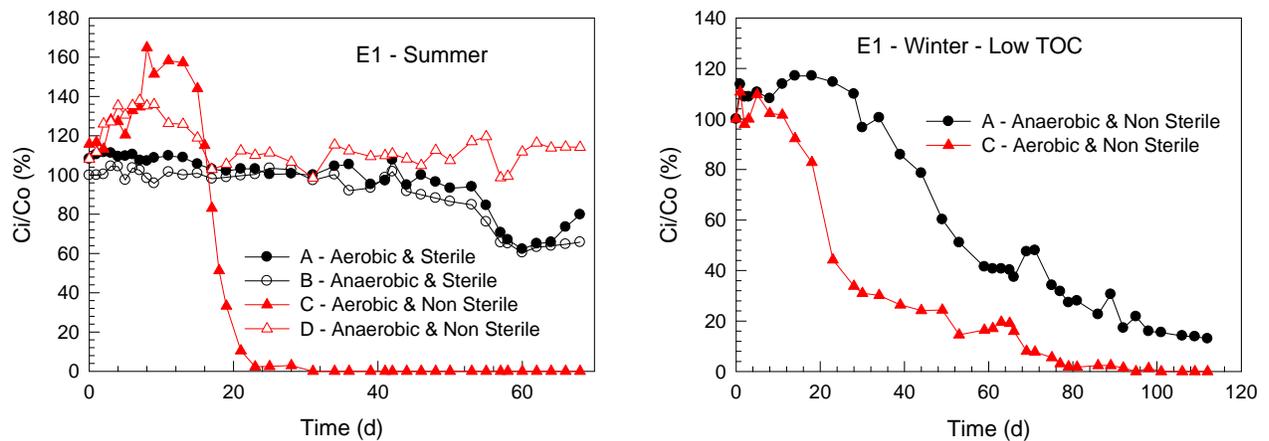


Figure 8. Impact of redox conditions and temperature on fate and transport of estrone (E1).

Publications Cited in Synopsis

- Caliman, F.A., and M. Gavrilesu, 2009, "Pharmaceuticals, personal care products and endocrine disrupting agents in the environment—A review," *Clean-Soil, Water, Air*, 37(4–5), 277–303.
- Hiscock, K.M., and T. Grischek, 2002, "Attenuation of groundwater pollution by bank filtration," *Journal of Hydrology*, 266(3–4), 139–144.
- Ray C., and M. Shamrukh, (eds.), 2011, *Riverbank Filtration for Water Security in Desert Countries*, NATO Science for Peace and Security Series C: Environmental Security, Springer, The Netherlands.
- Ray C., G. Melin, R.B. Linsky, (eds.), 2002, *Riverbank Filtration: Improving Source-Water Quality*, Kluwer Academic Publishers and National Water Research Institute, Boston.

Molecular, Fluorometric and Confocal Microscopy Analysis of Microbial Community Composition and Structure

Basic Information

Title:	Molecular, Fluorometric and Confocal Microscopy Analysis of Microbial Community Composition and Structure
Project Number:	2012HI368B
Start Date:	3/1/2012
End Date:	2/28/2013
Funding Source:	104B
Congressional District:	Hawaii 1st
Research Category:	Water Quality
Focus Category:	Acid Deposition, Ecology, Nutrients
Descriptors:	
Principal Investigators:	Celia Smith, David Spafford

Publications

There are no publications.

FINAL REPORT

**Molecular, Fluorometric and Confocal Microscopy
Analysis of Microbial Community Composition
and Structure in Sites Around Oahu**

May 2013

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Project Number: 2012HI368B

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Problem and Research Objectives

Biofilms are generally described as surface-associated bacterial communities surrounded by extracellular polymeric substances (EPS) [1] in aquatic or marine environments [2]. Most marine biofilms are composed of several different species that are probably arranged within the biofilm structure. It is generally known that EPS plays an important role in the adhesion of bacteria to surfaces and acts as “glue” and functions at cellular and intercellular levels [3,4]. Thus, EPS provide a nutrient-rich environment where bacteria can grow in complex biofilm communities. The diversity of marine microorganisms plays key roles in marine food webs and the cycling of nutrients. Marine biofilms have been shown to be associated with the settlement and marine sessile organisms such as invertebrates and algae [5].

Submerged surfaces are quickly covered by glycoconjugates and by microorganisms such as bacteria, diatoms and protozoa that form a biofilm [6]. The microbial communities in marine biofilms can have a strong influence on diatoms, algae and benthic invertebrates [7]. Indeed, marine bacteria can have stimulatory or inhibitory effects on the marine algal growth through the production of substances or lack of nutrients [8].

PCR-based molecular genetic techniques are very useful in studying microbial diversity, and DGGE, which separates PCR-amplified community 16S rDNA sequences. DNA bands from DGGE gels can be excised, and sequenced to identify community members. Thus, DGGE can be used to describe overall microbial diversity to identify individual community members from biofilm [9]. However, studies of the structure of biofilms require the intact biofilm remain on the surface. The biofilm structure can be studied three-dimensionally by Confocal Laser Scanning Microscopy (CLSM). This technique allows optical sectioning of the intact and undisturbed biofilm, and CLSM has been used in several studies for characterization of biofilm components and structure [10].

WRRC-sponsored research in 2012–2013 has allowed implementation of denaturing gradient gel electrophoresis (DGGE) to characterize bacteria in biofilms, with microscopic and fluorometric analyses. Biofilms are easily collected following immersion of substrates in shallow water sites; collection method in this study is easily modified to include deeper sites. Current efforts compare bacteria at shallow water eutrophic and oligotrophic field sites on Oahu via DGGE analysis, DNA sequence analysis, multidimensional scaling (MDS) and confocal microscopy characterizations to identify specific bacteria (e.g., fecal associated *Enterococcus* species and *Staphylococcus aureus*).

Applying methods in this study could allow to assess the microbial community associated with outfalls or sites with sewage spills on Oahu [11]. In an assessment of the microbial activity at the outfall, Novitsky and Karl [12] conclude “Although the activity of the microbial

populations does not seem to have been affected drastically by the input of sewage effluent, we have no indication of its effect on the microbial community structure. The fate of the large microbial community on the effluent itself is unknown.” This is a serious short-coming, as a shift in the microbial community structure can be pathological and has serious ecological implications [13], especially in regions where ocean activities are so popular.

The objective of this research was to examine biofilm communities from select sites on Oahu, including sites which have potentially elevated nutrients or impaired water inputs (e.g., Ala Wai Canal) and invasive alien algae (Waikiki and Maunalua Bay), as well as reference sites, to characterize if and how quickly microbial communities shift from impacted to healthy states. Study of microbial community composition within the biofilm can directly aid in the understanding of the health of our coastal marine ecosystem. Research in coastal regions complements those of CMORE with their more open ocean research themes (www.hawaii.edu/cmore).

Methodology

Study Sites. The experimental arrays on Oahu were deployed in a less-developed Hawaii Kai site (pristine site), at Waikiki alongside the Natatorium—a site dominated by invasive weed *Gracilaria salicornia*, at the McCully bridge crossing the Ala Wai, and at Hawaii Kai reef which is impacted with *Avrainvillea amadelpha*. The arrays of three replicate microscope slides (10 cm x 4 cm) were vertically positioned and suspended at 1 m depth.

Sampling. Biofilm samples on each array were scraped by a sterile razor blade after 7 days immersion and kept in a 2 ml microfuge tube. Samples were stored in a -80°C freezer for future analysis. For image analysis, a glass slide was kept in a coplin jar with seawater collected from each study site, and observed or preserved in 2% formalin for microscopy.

Genomic DNA Extraction and PCR Amplification. Total genomic DNA were extracted from the scraped biofilm samples using a Powerbiofilm DNA kit (MB BIO Laboratories, Inc.). The extracted DNA (dissolved in 10 mM Tris-HCl, 1 mM EDTA, pH 7.5) were stored at -80°C until further analysis. PCR mixtures (50 µl total volume) contained 200 µM deoxynucleoside triphosphates (dNTPs), 1 µM of each primer, 0.05 unit/µl Taq DNA Polymerase, and 100 ng genomic DNA as template (negative controls with water). Partial 16S rDNA gene amplifications for DGGE analysis were performed using the universal primer sets for bacteria [14, 15]. The PCR program included an initial denaturation at 94°C for 5 min, denaturation at 94°C for 30 sec, annealing at 57°C for 1 min, and extension at 72°C for 1 min, followed by 25 cycles. The PCR

products were visually analyzed by electrophoresis on 2% agarose gels (w/v) and run in 1 x TAE buffer stained with ethidium bromide (0.5 mg/l).

DGGE Analysis. DGGE analysis was performed with a DGGE-2000 system (C.B.S. Scientific Co.). The condition of DGGE was performed with 0.75-mm-thick 6% polyacrylamide gels (ratio of acrylamide to bis-acrylamide, 37.5:1) submerged in 0.5 x TAE buffer (40 mM Tris, 40 mM acetic acid, 1 mM EDTA; pH 7.4) at 60°C. The PCR samples were mixed with 6 µl of dye solution (0.1% bromphenol blue [w/v], 70% glycerol [v/v]) and applied to the gels. Electrophoresis was performed during 12 h at 30 V in a linear 20 to 60% denaturant agent gradient. That 100% denaturant agent was defined as 7 M urea with 40% formamide. The gels stained for 30 min in 1 x TAE buffer with SybrGold nucleic acid stain (Molecular Probes) and visualized with UV radiation used by Bio Photometer (Eppendorf) and Kodak Scientific Imaging Systems. The distinct and dominant 16S rDNA bands from the results of DGGE were excised from multiple lanes of the gels by a sterilized razor blade. Excised DNA bands were re-suspended in 0.5 ml of MilliQ water, and stored at 4°C overnight [16]. Re-suspended DNA re-amplified PCR reaction and duplicate amplifications of DGGE for DNA sequence analysis is described above.

Nucleotide Sequencing. All re-amplification products of the DGGE bands were sequenced directly in both directions using the respective amplification primers and the ABI Prism BigDye terminator cycle sequencing ready reaction kit (Applied Biosystems, Foster City, USA). Sequencing reactions were run on a ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems).

Phylogenetic Analysis. The partial 16S rDNA sequences obtained from DGGE analysis were checked for chimeras with the program CHECK_CHIMERA of the Ribosomal Database Proposal and added to the MEGA-3 software program. Each set of sequences was grouped into phylotypes, (operational taxonomic units [OTUs]), based on a > 97% identity cutoff. The closest phylogenetic relatives of each phylotype were identified by comparison to the National Center for Biotechnology Information (NCBI) GenBank database using the Basic Local Alignment Search Tool (BLAST) analysis tools (www.ncbi.nlm.nih.gov/BLAST/).

Sequence analysis of cultured biofilm bacteria. Bacteria from the Ala Wai biofilm samples were inoculated on Tryptic Soy Agar (TSA) media and grown at room temperature for 48 h. DNA was extracted from isolated colonies and amplified 16s rDNA as in 'genomic DNA extraction and PCR amplification' protocol. Amplified 16s rDNA samples were identified by nucleotide sequencing and compared to the NCBI GenBank database using the BLAST analysis tools.

Pulse Amplitude Modulated Fluorometry (PAM). PAM (using the Junior PAM device, Waltz, Germany) was used to assess the photosynthetic characteristics of autotrophic organisms

attached to glass slides at the four research study sites. We examined if and to what extent the study site were changing the microbial community by measuring the differences in photosynthesis. PAMs rapid light curves (RLC) were performed to determine the maximum electron transport rate (ETR_{max}) of photosystem II (PS II) of the sampled organisms [17]. All samples were continuously immersed in seawater after collection. RLCs were performed in shade immediately after collection. For each of the four study sites, three replicate RLCs were performed on each of three randomly selected slides (9 RLCs/site). The mean ETR_{max} for each site was calculated.

Microscopy. Biofilms were visualized with light and CLSM. The data presented here focused on biofilms from the Ala Wai site that were grown on glass slides in the same manner as for DGGE analysis. To support this work we inoculated biofilm scrapings onto microbial media including TSA, EMB, and/or MSA; incubated for 48 h at room temperature; and isolated colonies for study. Gram and/or capsule stains were carried out directly after isolating the colonies.

To visualize the cells and EPS/biofilm matrix on the glass slides, we stabilized glycoconjugates and stained biofilms with fluorescently-labeled lectins after the methods of Michael and Smith 1995 [18]. Lectins were chosen for their affinities in complementary binding with mannose/glucose moieties (Concavalan A [Con A]) or N-acetylglucosamine and sialic acid residues (Wheat Germ Agglutinin [WGA]). Commercially prepared lectins with fluorescein isothiocyanate (FITC) were obtained from Vectra Laboratories (Vectra Laboratories Inc., Burlingame, CA, USA). The FITC-labeled lectins were dissolved in a stock solution containing 10 mM HEPES, 0.15 M NaCl, pH 7.5, 0.1 mM Ca⁺⁺, and 0.08% sodium azide (0.01 mM Mn⁺⁺ for Con A). Microbes were visualized with the use of 4',6-diamidino-2-phenylindole (DAPI) to localize DNA. Labeled biofilms were examined with an Olympus FV1000 Laser Scanning Confocal Microscope with digital image capture. Exciting lasers were 488 nM for FITC channel, 543 nM for red autofluorescence (Rhodamine channel), and 405 nM for the DAPI channel (Blue Diode).

Water Quality Analysis. Water samples from each site were collected in a 50 ml test tube by 0.2 µm filtration. The samples were analyzed using an Exeter Elemental Analyzer for nitrogen and sulfur analysis (CNS), via the new analytical laboratory in SOEST.

Principal Findings and Significance

To analyze changes of marine microbial communities on the effect of bacteria at impacted urban reefs compared to a pristine site (Hawaii Kai), the DGGE fingerprint technique was

applied using the universal primer sets (GM5F-GC clamp 341F and 907R). This was done in order to obtain a partial 16S rDNA gene, to analyze the 16S rDNA sequence from the pristine site, and finally to compare that community with suspected impacted sites (Waikiki, Ala Wai Canal, and Hawaii Kai in the *Avrainvillea amadelpha* meadow) on Oahu (Figure 1).

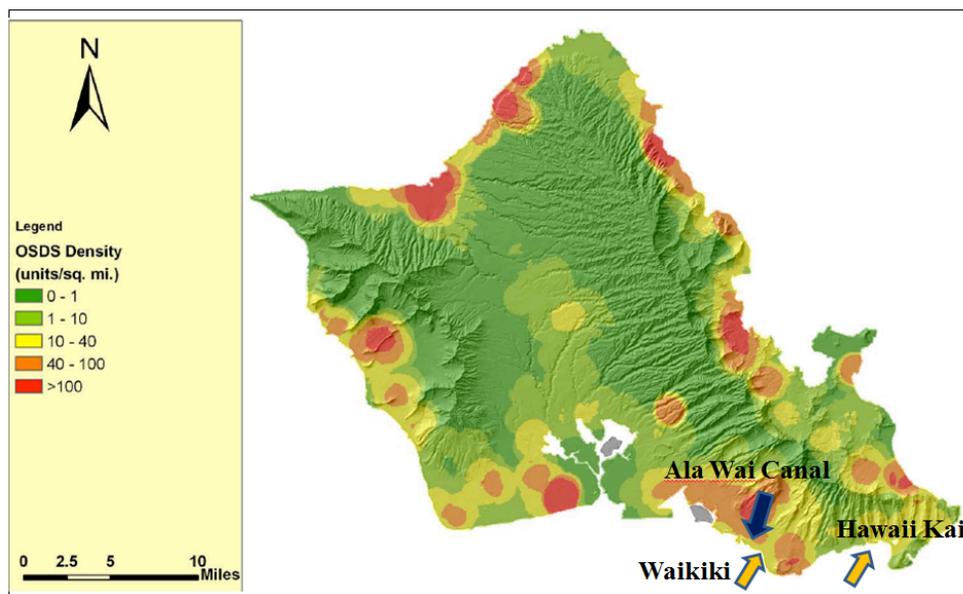


Figure 1. Study sites on Oahu based on Onsite Sewage Disposal System (OSDS) report [from 19].

DGGE results indicated that a total of 57 different 16S rDNA patterns were detected from the study sites (Figure 2). The dominant and distinct DGGE fragments were excised and identified for sequence analysis (Figure 4). To investigate the intensity matrix from the DGGE bands study sites, the present study used MDS. Different DGGE fingerprints are shown in Figure 3. Encircled areas indicate that the compositions of the microbial communities were significantly distinct at each of the study sites. The compositions of microbial communities from Hawaii Kai reef were clearly distinguished from the other study sites within 12.8% of similarity (Figure 3A and B). On the other hand, the compositions of microbial communities between Waikiki and Ala Wai Canal showed 37.7% similarity; the study areas that are geographically close share aspects of the environment that similarly influence the microbiota.

To assess differences of microbial community per study site, a pair-wise test was used for an analysis of similarities function (ANOSIM). The results showed R significance level (%) for the composition difference in microbial communities based on the different sites (Table 1). The present study interpreted that R-values > 0.75 as well distinguished among groups; R > 0.5 as overlapping, but clearly different among groups; and R < 0.25 as barely separable at all, in accordance with the Primer 6 manual [19]. According to results of our study, global R value

0.796 indicated that the compositions of microbial communities showed significantly different results based on the different sites during the experimental period. In addition, the stress values in MDS (Figure 3A) showed 0.1 that indicates an excellent representation with no prospect of misinterpretation within 0.5 levels [20]. Therefore, the results strongly indicate the compositions of microbial communities are distinct among the marine microbial community at these different study sites. While the community composition has largely unknown effects on upper trophic levels, they are likely to be very important to overall ecosystem health. Further, this tool could lead to rapid assessment of the different microbial consortia that could be found in other fine scale sampling, such as regions adjacent to outfalls or sites with chronic sewage spills as in Ala Wai Canal.

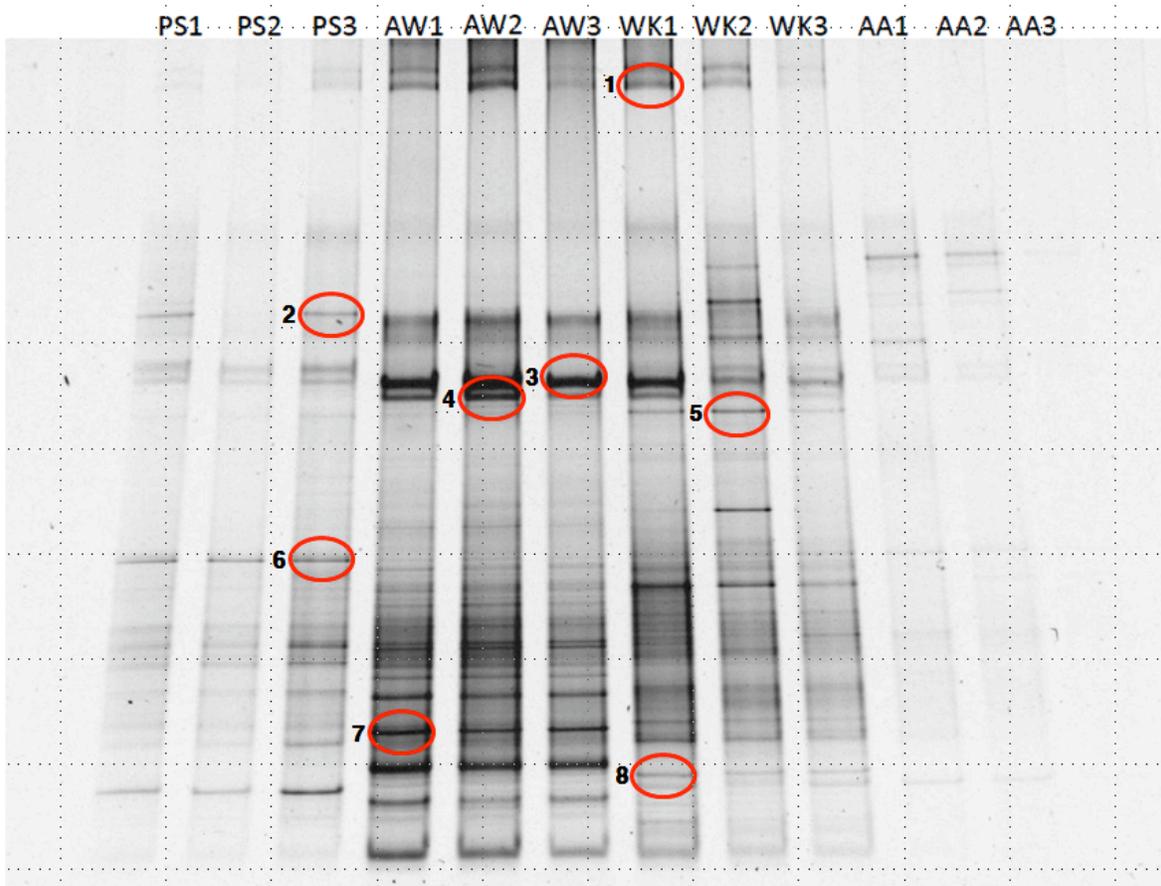


Figure 2. Images of DGGE fingerprint profiles containing 16S rDNA fragments which were amplified with universal primer sets (GM5F-GC clamp 341F and 907R) from the Hawaii Kai pristine site and the three impacted sites (Ala Wai Canal, Waikiki, and the *Avrainvillea* meadow in Hawaii Kai reef) on Oahu. Microbial samples with three replicates were scraped by a sterile razor blade after 7 days. The numbered red circles refer to the excised and identified bands for DNA sequence analysis (Figure 4).

Table 1. Pairwise test by the analysis of similarities function (ANOSIM) among groups. The sample statistic (Global R) for all pairwise data was 0.796.

Pairwise Field Sites	R Significance Level
Pristine, Ala Wai	1
Pristine, Waikiki	1
Pristine, Hawaii Kai	0.833
Ala Wai, Waikiki	1
Ala Wai, Hawaii Kai	1
Waikiki, Hawaii Kai	0.648

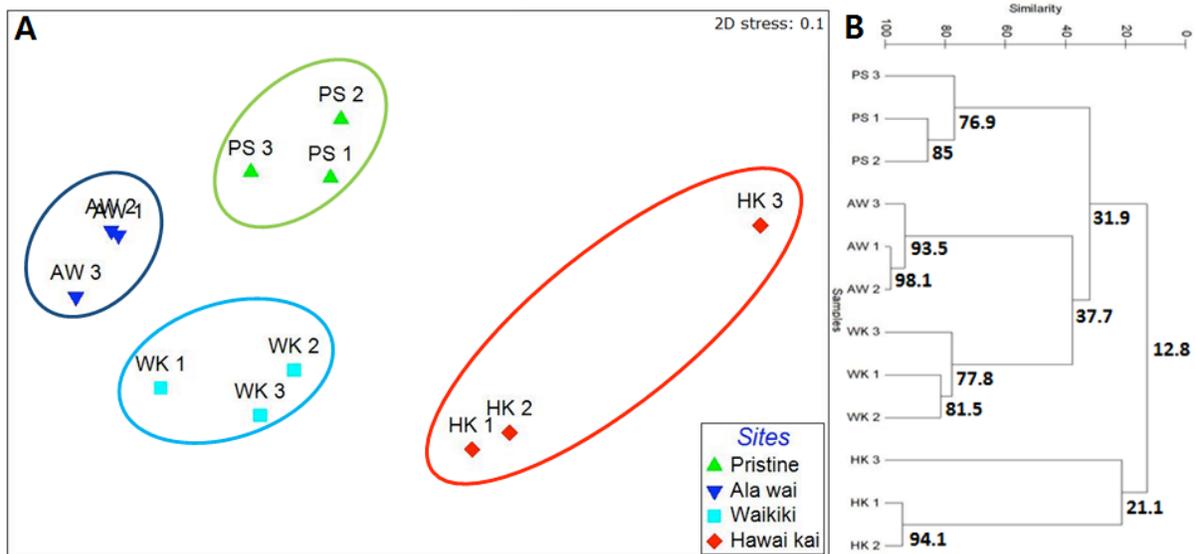


Figure 3. (A) Multidimensional scaling (MDS) diagram representing the changes in bacterial community structure at impacted urban reefs compared to a pristine site. Encircled areas indicate groups that showed high similarity among samples. (B) The scale bar indicates the percent of similarity among groups.

For DNA sequence analysis based on the excised DGGE bands (Figure 2), a total of 8 distinct DGGE fragments were excised and identified from the study sites (Figure 4). Among diversely revealed bacterial communities during the experimental period, *Synechococcus* sp. (CC9311, Figure 2, Band #1) was detected on all study sites except at Hawaii Kai reef. *Synechococcus* sp. is one of oxygenic photoautotrophs of the genera and widely accepted as the most abundant members of the picophytoplankton that contributes significantly to primary production in the ocean [21].

Two distinct DGGE fragments were excised and identified from the Hawaii Kai's pristine site: *Shewanella denitrificans* (OS217, Figure 2; Band #2) and *Saprospira grandis* (str. Lewin, Figure 2; Band #6). *S. denitrificans* (OS217, Figure 2; Band #2) is a diverse group of marine gamma-proteobacteria. It is an important species because *S. denitrificans* contributes significantly to the total denitrification of the system in the ocean [22]. *S. grandis* (str. Lewin, Figure 2; Band #6) is a gram-negative marine bacterium that is free-living in marine littoral sand or coastal zones. *S. grandis* contributes to consumption of algae in the prevention of algal blooms [23, 24]. Additionally, Sangkhobol et al. [25] reported *S. grandis* also preys on other bacteria and protists.

Three distinct DGGE fragments were excised and identified from Ala Wai Canal: *Marivirga tractuosa* (DSM 4126, Figure 2, Band #3), *Flavobacterium johnsoniae* (UW101, Figure 2, Band #4), and *Vibrio vulnificus* (CMCP6, Figure 2, Band #7). *M. tractuosa* (DSM 4126, Figure 2, Band #3) is a gram-negative bacterium that is non-spore forming. *M. tractuosa* (DSM 4126) is found in a variety of places such as soil, freshwater or beach sand. *F. johnsoniae* (UW101, Figure 2, Band #4) is an aerobic gram-negative bacterium that is commonly found in soil and freshwater and rapidly digests chitin and many other macromolecules [26]. The last distinct DGGE fragment from Ala Wai Canal is *V. vulnificus* (CMCP6, Figure 2, Band #7) which is a gram-negative bacterium and found in salty or coastal waters. It can thrive in molluscan shellfish such as oysters, thereby causing health risks for those who ingest raw or undercooked seafood. Thompson et al. [27] reported *V. vulnificus* can be a virulent bacterium associated with about 95% of all seafood related deaths.

From Waikiki, two distinct DGGE fragments were excised and identified which were *Synechococcus* sp. (PCC7002, Figure 2, Band #5) and *Pseudomonas mendocina* (ymp, Figure 2, Band #8). *Synechococcus* sp. was found in a DGGE gel band from the Hawaii Kai pristine site, as well as in Ala Wai Canal, and in Waikiki. It seems to indicate that the equal 16S rDNA sequences could present at different positions on the DGGE gel. On the other hand, Boon et al. [28] and Vallaeys et al. [29] reported that a single DGGE fragment could be interpreted by numerous species with partial 16S rDNA sequences. *P. mendocina* is a gram-negative bacterium that can be found in many different environments such as soil, marshes, and coastal marine habitats. *P. mendocina* can cause opportunistic nosocomial infections, such as infective endocarditis [30].

DNA sequence analysis of 4 colonies isolated from Ala Wai samples indicate that *Shewanella baltica* (homology: 89%), *Vibrio cholerae* IEC224 (homology: 91%), *Pseudomonas syringae* B728 (homology: 97%) and *Escherichia coli* MG1655 (homology: 100%) were found as biofilm components at this site. Notably, *Shewanella* sp., *Vibrio* sp., *Pseudomonas* sp. had

similar DGGE fingerprint results. These results indicate that the microbial community structure from the study sites can be pathological and could have serious ecological implications.

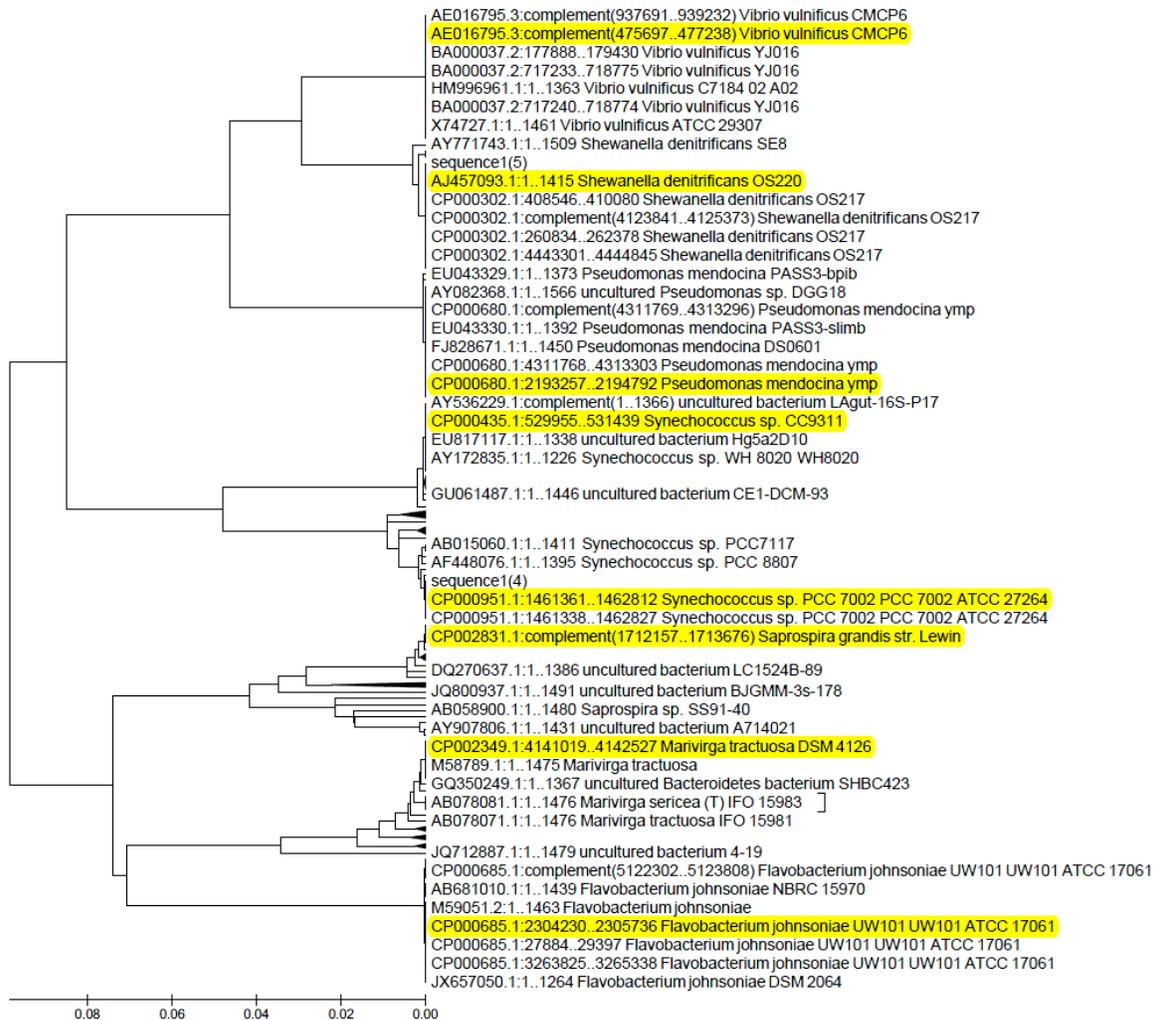


Figure 4. Phylogenetic tree based on DGGE fingerprint profiles from the microbial community in the test sites. The yellow bands indicate organisms we identified within our study.

Biofilm Microscopy for Community Structure

Light microscopy of biofilm slides from the Ala Wai showed gram positive and negative bacteria with notable components including marine yeasts (to be confirmed), diverse capsulated microbes (Figures 5 and 6), and endospores or endospore forming bacteria (Figure 7).

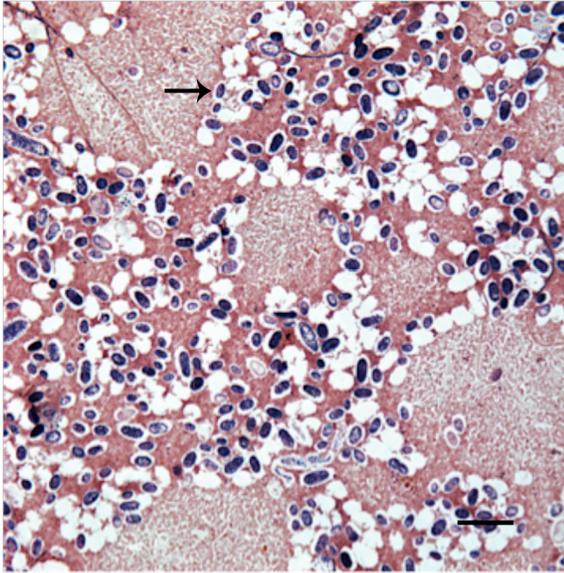


Figure 5. Gram-negative bacteria that grew on an EMB medium but did not ferment lactose (non-coliforms). These bacteria were interspersed with large, capsulated (arrow) eukaryotic cells on the glass substrate we speculate are marine yeasts. Scale 100 μm .

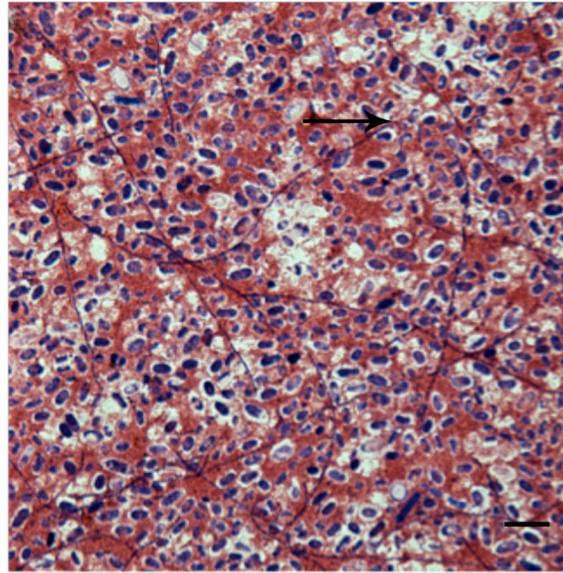


Figure 6. The "yeast" and gram-negative bacteria were consistently associated with filamentous bacteria (arrow) that were also evident in this biofilm community. Scale 50 μm .

Biofilms were inoculated onto TSA plates, grown at room temperature for 48 hours and colonies were isolated. A gram-positive bacterium was found to produce endospores during this incubation time (Figure 7). While capsules in marine microbes are common and diverse, the presence of capsules for several bacteria isolated in our study, withstood gram stain conditions. The capsules as well as endospores and/or endospore-forming organisms in the biofilms from Ala Wai could be considered as pathogenic potential for these organisms. The overall dimensionality of a biofilm is shown by Differential Interference Contrast Microscopy (DIC) (Figure 8) and further evaluated with confocal microscopy.

To evaluate the community structure we used lectins to stabilize the biofilms that were conjugated with FITC for visualization. Biofilms were additionally labeled with DAPI for visualization of biofilm microbes.

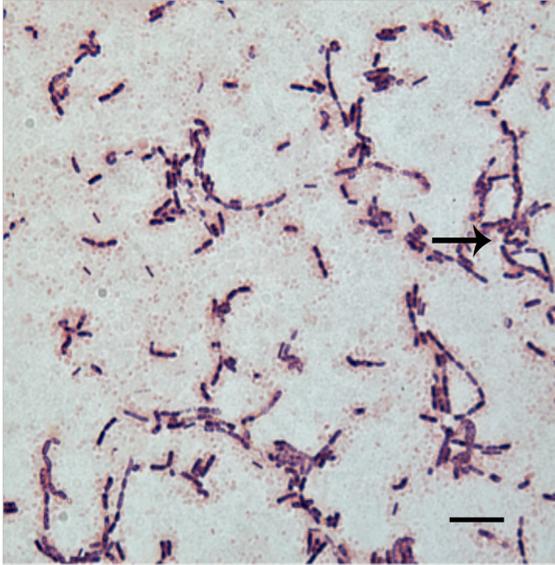


Figure 7. Gram positive bacteria and endospores (arrow) following inoculation onto Ala Wai biofilm organisms TSA and incubation at room temperature. Scale 10 μm .

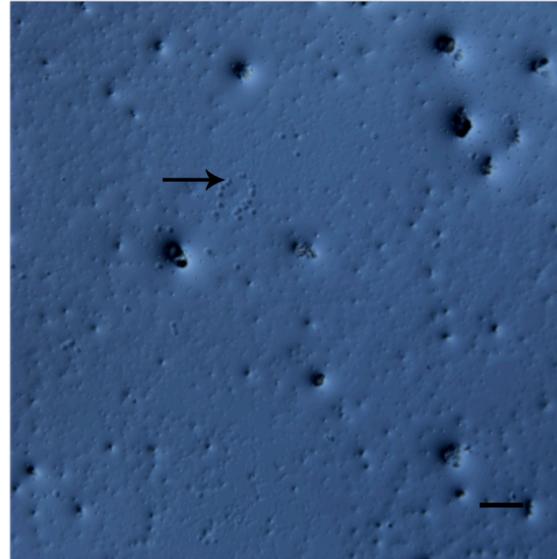


Figure 8. DIC overview of an unstained biofilm showed scattered cocci (small dots) and larger eukaryotic cells (arrow) that were determined to be photosynthetic with epifluorescence excitation (green). Scale 50 μm .

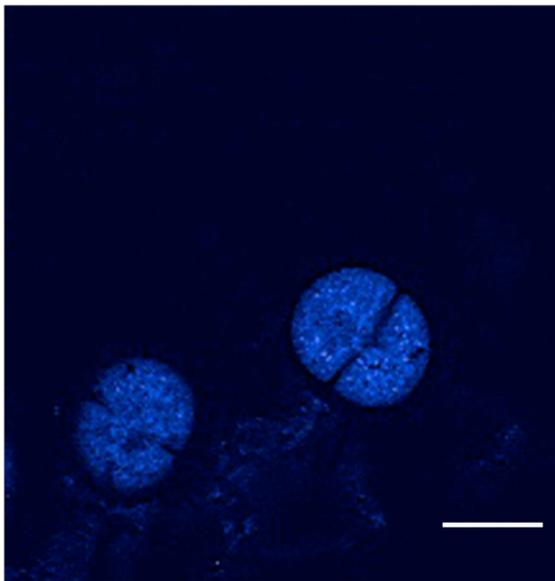


Figure 9. DAPI-stained photosynthetic cells in the biofilm were associated with EPS. Scale 25 μm .

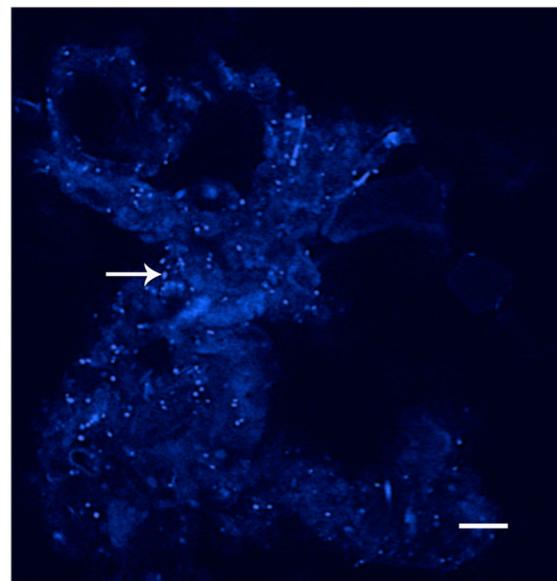


Figure 10. DAPI-stained bacteria (arrow) were within EPS of the biofilm. Scale 50 μm .

The consistent associations of biofilm cells and EPS (Figures 9 and 10) allow us to see glycochemistry sources and distribution, and we further visualized the glycochemistry of the biofilms with the use of lectins.

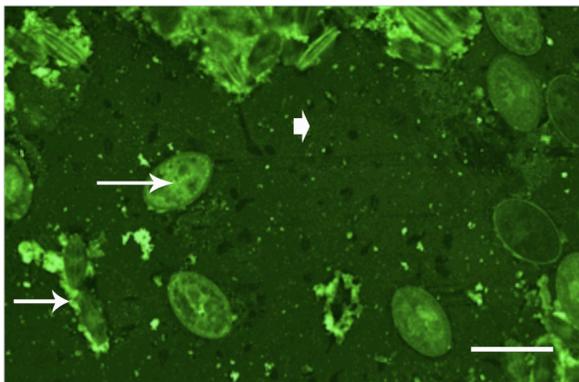


Figure 11. Con A-labeled biofilm shows mannose/glucose-rich glycoconjugates from diatom EPS (arrows) and non-cellular matrix (arrowhead). Scale 50 μm .

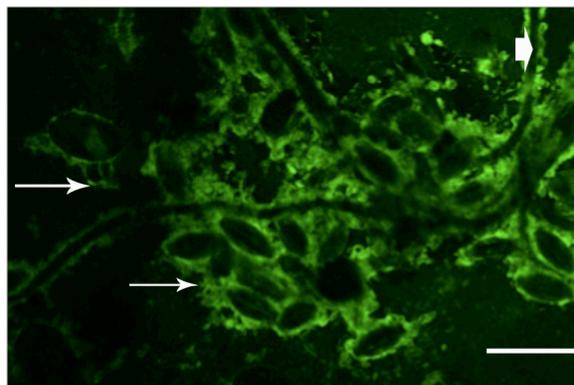


Figure 12. WGA-labeled EPS from diatoms (arrows) and cyanobacteria (arrowhead) localized glycoconjugates with N-acetylglucosamine residues external to the cells as EPS. Scale 50 μm .

Our results indicate complex chemistry of the EPS from diatoms and bacterial cells/EPS. By the specificity of the lectin binding we concluded that the composition of the biofilm matrix contains glycoconjugates with mannose/glucose moieties which is also localized at bacterial cells and EPS (Figure 11). The glycoconjugates with sialic acid residues are most concentrated in the diatom and bacterial EPS (Figure 12). The community structure of Ala Wai biofilms has been visualized with diverse organisms that include photoautotrophs (Figures 13 and 14). Specific glycoconjugates reveal remarkable complexity in the biofilm matrix and EPS (Figures 11–14). The distribution of specific cellular and EPS glycoconjugates has significance in substrate colonization as well as future community development in this coastal region [31].

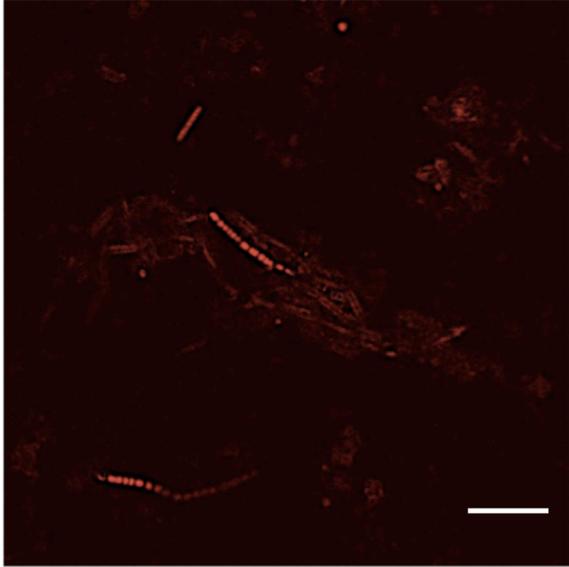


Figure 13. Photosynthetic components of the biofilm were evident as red autofluorescence following green laser excitation. Scale 100 μm .

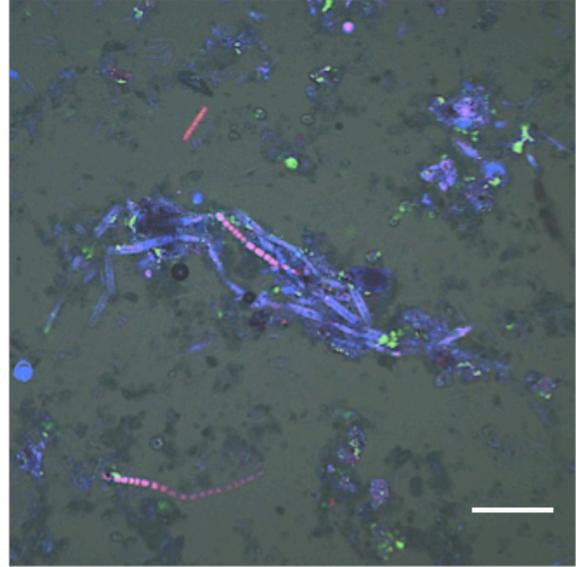


Figure 14. This composite image from excitation with blue, green and UV lasers as well as white light, illustrates cellular and acellular components of the biofilm structure with heterogeneous distribution of mannose/glucose specific glycoconjugates. The autofluorescent cyanobacteria (red) and diatoms and bacteria (blue) as well as mannose/glucose-rich glycoconjugates (green) in the matrix and EPS structure the biofilm community. Scale 100 μm .

Water Quality Analysis

The results of the inorganic nutrient levels for the water quality analysis are shown in Table 2. Ala Wai Canal has high levels of inorganic nutrients among the study site. In contrast, the pristine site shows the lowest levels of inorganic nutrients among the study site. The Ala Wai Canal is eutrophic via inputs of impaired water or with sewage spills and this has been a public health concern.

Table 2. Results of the inorganic nutrient level from the study sites (unit: $\mu\text{mol/L}$).

Site	Nitrate	Nitrite	Inorganic P	Silicate	Ammonia	Nitrate
Pristine reef	1.76	0.067	0.014	3.21	1.22	1.70
Ala Wai Canal	7.86	0.463	0.390	74.54	1.62	7.40
Waikiki reef	1.17	0.111	0.122	2.11	0.327	1.06
Hawaii Kai reef	2.90	0.050	0.074	2.23	4.10	2.85

To examine the photosynthetic characteristics of autotrophic organisms attached to the glass, PAM was used to measure the differences of photosynthesis in the microbial community among the four study sites (Figure 15). The results indicate that Ala Wai Canal showed the highest level of ETRmax at 35.9, while Hawaii Kai reef showed the lowest level at 16.7. The data indicates the population of autotrophic organisms in the Ala Wai Canal is higher than other study sites. In addition, the results of the ETRmax t-test showed $P = 0.044089$ and that the data of ETRmax are significant at < 0.05 (Excel).

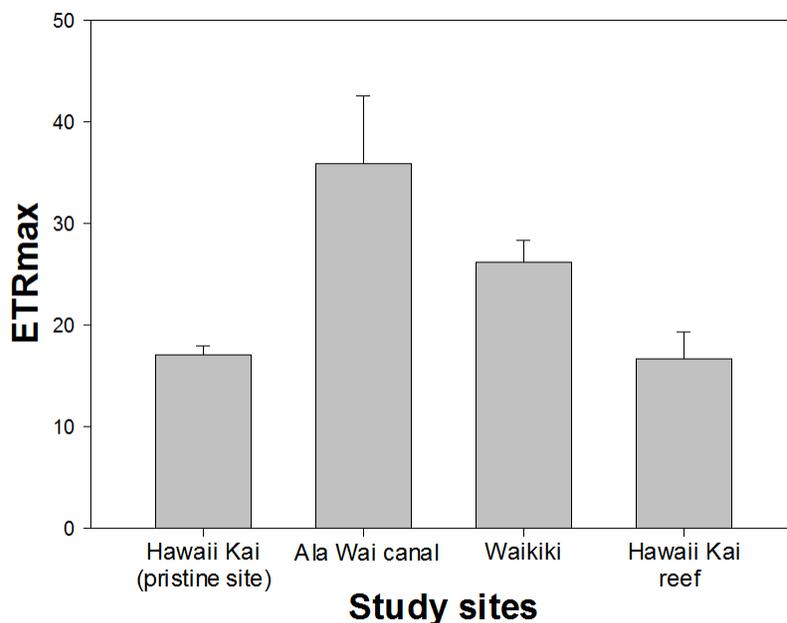


Figure 15. Mean values (+ standard deviation) of ETRmax for biofilms at the four study sites.

Our examination of Oahu's biofilm microbial communities including those that have potentially elevated wastewater inputs, especially for Ala Wai Canal and Waikiki, showed pathological and potentially serious ecological implications in the findings with organisms such as *Vibrio vulnificus* and *Pseudomonas mendocina*. How microbial communities quickly shift from impacted invasive alien algae to healthy states are still unknown. The results of this study provide information on the microbial community composition within the biofilm that could become one of research priorities in the understanding of marine ecosystems and effects of elevated wastewater inputs.

Conclusion and Potential Significance of this Work

To analyze changes in marine microbial communities, bacteria at impacted urban reefs were compared to a pristine site with DGGE analysis. Our results indicated that a total of 57 different 16S rDNA patterns were detected from the study sites. The compositions of microbial communities from Hawaii Kai reef were clearly distinguished from the other study sites within 12.8% of similarity. On the other hand, the compositions of microbial communities between Waikiki and Ala Wai Canal showed 37.7% similarity. Therefore, the results strongly indicate the compositions of microbial communities are distinct among the marine microbial community at these different study sites, and at relatively fine scales.

For DNA sequence analysis based on the excised DGGE bands, a total of 8 distinct DGGE fragments were excised and identified from the study sites. A diverse group of bacterial communities was also found in the study sites during the experimental period (*Synechococcus* sp. CC9311, *Shewanella denitrificans* OS217, *Saprospira grandis*, *Marivirga tractuosa* DSM 4126, *Flavobacterium johnsoniae* UW101, *Vibrio vulnificus* CMCP6, *Synechococcus* sp. PCC7002, and *Pseudomonas mendocina* ymp). *S. denitrificans* OS217, *V. vulnificus* CMCP6, and *P. mendocina* ymp indicate that the microbial community structure from the study sites can be pathological and could have serious ecological implications.

The biofilm microscopy for community structure results indicated complex chemistry of the EPS from diatoms and bacterial cells/EPS. The specificity of the lectin binding allows us to conclude that the composition of the biofilm matrix contains glycoconjugates with mannose/glucose moieties that is also localized at bacterial cells and EPS. The glycoconjugates with sialic acid residues are most concentrated in the diatom and bacterial EPS.

Results of water chemistry and PAM indicated that Ala Wai Canal showed high levels of inorganic nutrients and the highest algal photosynthesis level (ETR_{max}) among the study sites.

Therefore, we could conclude Ala Wai Canal is eutrophic via inputs of impaired water or with sewage spills, and the populations of autotrophic organisms are higher than at other study sites.

On Oahu, including sites that have potentially elevated wastewater inputs, indications of pathological and serious ecological implications were found (e.g., *Vibrio vulnificus* and *Pseudomonas mendocina*) in the microbial community structure, especially at Ala Wai Canal and at Waikiki. However, how quickly microbial communities shift from impacted invasive alien algae to healthy states, remains unknown.

References Cited

- [1] Costerton, J.W., Z. Lewandowski, 1995, Microbial biofilms, *Annual Review of Microbiology*, 49, 711–745.
- [2] Kristensen, J.B., R.L. Meyer, B.S. Laursen, S. Shipovskov, F. Besenbacher, C.H. Poulsen, 2008, Antifouling enzymes and the biochemistry of marine settlement, *Biotechnology Advances*, 26, 471–481.
- [3] Lawrence, J.R., D.R. Korber, B.D. Hoyle, J.W. Costerton, D.E. Caldwell, 1991, Optical sectioning of microbial biofilms, *Journal of Bacteriology*, 173, 6558–6567.
- [4] Wetherbee, R., J.L. Lind, J. Burke, R.S. Quatrano, 1998, Mini review—The first kiss: establishment and control of initial adhesion by raphid diatoms, *Journal of Phycology*, 34, 9–15.
- [5] Egan, S., T. Thomas, S. Kjelleberg, 2008, Unlocking the diversity and biotechnological potential of marine surface associated microbial communities, *Current Opinion in Microbiology*, 11, 219–225.
- [6] Little, B.J., J.R. DePalma, 1998, Marine biofouling, in D.F. Hanson, C.R. Crowe, H. Herman, eds., *Treatise on Materials Science and Technology-Materials for Marine Systems and Structures*, New York, Academic Press, 89–119.
- [7] Salvesen, I., K.I. Reitan, J. Skjermo, G Øie, 2000, Microbial environments in marine larviculture: impacts of algal growth rates on the bacterial load in six microalgae, *Aquaculture International*, 8, 275–287.
- [8] McBain, A.J., 2009, *In vitro* biofilm models: an overview, in S. Sima, M.G. Geoffrey, eds., *Advances in Applied Microbiology*, vol. 69, Academic Press, 99–132.

- [9] Welsh, A.K., R.J. McLean, 2007, Characterization of bacteria in mixed biofilm communities using denaturing gradient gel electrophoresis (DGGE), *Current Protocols in Microbiology*, Chapter 1:Unit 1E.1.
- [10] Bjørkøy, A., L. Fiksdal, 2009, Characterization of biofouling on hollow fiber membranes using confocal laser scanning microscopy and image analysis, *Desalination*, 245, 474–484.
- [11] Hunt, C., 2007, Ground-water nutrient flux to coastal waters and numerical simulation of wastewater injection at Kihei, Maui, Hawaii, *U.S. Geological Survey Scientific Investigations Report 2006–5283*, 88.
- [12] Novitsky, J., D. Karl, 1985, Influence of deep ocean sewage outfalls on the microbial activity of the surrounding sediment, *Applied and Environmental Microbiology*, 50, 1464–1473.
- [13] Smith, J., M. Shaw, R. Edwards, D. Obura, O. Pantos, E. Sala, S.A. Sandin, S. Smriga, F. Rohwer, 2006, Indirect effects of algae on coral: algae-mediated, microbe-induced coral mortality, *Ecology Letters*, 9, 835–845.
- [14] Muyzer, G., A. Teske, C.O. Wirsen, H.W. Jannasch, 1995, Phylogenetic relationships of *Thiomicrospira* species and their identification in deep-sea hydrothermal vent samples by denaturing gradient gel electrophoresis of 16S rDNA fragments, *Archives Microbiology*, 164, 165–172.
- [15] Muyzer, G., 1999, DGGE/TGGE a method for identifying genes from natural ecosystems, *Current Opinion in Microbiology*, 28, 317–322.
- [16] Di'ez, B., C. Pedro-Alio, T.L. Marsh, R. Massana, 2001, Application of denaturing gradient gel electrophoresis (DGGE) to study the diversity of marine picoeukaryotic assemblages and comparison of DGGE with other molecular techniques, *Applied and Environmental Microbiology*, 67, 2942–2951.
- [17] Schreiber, U., R. Gademann, P.J. Ralph, A.W. Larkum, 1997, Assessment of photosynthetic performance of *Prochloron* in *Lissoclinum patella* in hospite by chlorophyll fluorescence measurements, *Plant and Cell Physiology*, 38, 945–51.
- [18] Michael, T., C.M. Smith, 1995, Lectins probe molecular films in biofouling: Characterization of early films on non-living and living surfaces, *Marine Ecology Progress Series*, 119, 229–236.

- [19] Whittier, R.B., A.I. El-Kadi, 2009, Human and environmental risk ranking of onsite sewage disposal systems, Department of Geology and Geophysics, University of Hawai'i at Manoa, Honolulu, HI. 57 pp.
- [20] Clarke, K.R., R.M. Warwick, 2001, Change in marine communities: an approach to statistical analysis and interpretation, 2nd ed., PRIMER-E, Plymouth.
- [21] Scanlan, D.J., N.J. West, 2002, Molecular ecology of the marine cyanobacterial genera *Prochlorococcus* and *Synechococcus*, *FEMS Microbiology Ecology*, 40, 1–12.
- [22] Brettar, I., E.R.B. Moore, M.G. Höfle, 2001, Phylogeny and abundance of novel denitrifying bacteria isolated from the water column of the Central Baltic Sea, *Microbial Ecology*, 42, 295–305.
- [23] Delk, A.S., C.A. Dekker, 2012, Characterization of rhabdosomes of *Saprospira grandis*, *Journal of Molecular Biology*, 64, 287–288.
- [24] Lewin, R.A., 2012, *Saprospira grandis*: a flexibacterium, *Microbial Ecology*, 34, 232–236.
- [25] Sangkhobol, V., V.B.D. Skerman, 2012, *Saprospira* species-natural predators, *Microbiology*, 5, 169–174.
- [26] Stanier, R.Y., 1947, Studies on non-fruiting myxobacteria, *Journal of Bacteriology*, 53, 297–315.
- [27] Thompson, F., B. Austin, J. Swings, eds., 2006, The biology of *Vibrios*, Hemdon, Virginia, ASM Press, 349–354, 359–361.
- [28] Boon, N., W.D. Windt, W. Verstraete, E.M. Top, 2002, Evaluation of nested PCR-DGGE (denaturing gradient gel electrophoresis) with group-specific 16S rRNA primers for the analysis of bacterial communities from different wastewater treatment plants, *FEMS Microbiology Ecology*, 39, 101–112.
- [29] Vallaey, T., E. Topp, G. Muyzer, V. Macheret, G. Laguerre, A. Rigaud, G. Soulas, 1997, Evaluation of denaturing gradient gel electrophoresis in the detection of 16S rDNA sequence variation in rhizobia and methanotrophs, *FEMS Microbiology Ecology*, 24, 279–285.
- [30] Aragone, M.R., D.M. Maurizi, L.O. Clara, E.J.L. Navarro, A. Ascione, 1992, *Pseudomonas mendocina*, an environmental bacterium isolated from a patient with human infective endocarditis, *Journal of Clinical Microbiology*, 30, 1583–1584.
- [31] Michael, T.M., 2009, Glycoconjugate organization of *Enteromorpha* (= *Ulva*) *flexuosa* and *Ulva fasciata* (Chlorophyta) zoospores, *Journal of Phycology*, 45, 660–677.

Acquire Sedimentation Data to Promote Reservoir Sustainability and Advance Watershed Science

Basic Information

Title:	Acquire Sedimentation Data to Promote Reservoir Sustainability and Advance Watershed Science
Project Number:	2012HI370B
Start Date:	3/1/2012
End Date:	2/28/2014
Funding Source:	104B
Congressional District:	Hawaii 1st
Research Category:	Water Quality
Focus Category:	Geomorphological Processes, Management and Planning, Sediments
Descriptors:	
Principal Investigators:	David C. Penn

Publications

There are no publications.

Problem and Research Objectives

Maintaining and increasing reservoir storage capacity is an adaptive measure for potential climate change impacts that also supports drought resilience and contributes to island food security and stormwater reclamation efforts. However, Hawaii's reservoirs face growing scrutiny due to heightened dam safety and flood control concerns, changing water demands, and uncertain water pollution effects. As a result, over 15% of Hawaii's regulated dam structures appear to be slated for decommissioning. Many of our remaining reservoirs show signs of significant sedimentation, accompanied by a wide range of management responses.

Sediment deposition in reservoirs is an episodically continual process, and leaving issues unaddressed until problems become acute often leads to costly, rushed, and/or ineffective solutions (Randle et al., 2013). In order to promote reservoir sustainability, it is vital that we improve our understanding of reservoir capacity loss due to sedimentation. Although the National Inventory of Dams (NID) and the State of Hawaii Dam Inventory each include records for about 140 reservoirs (Figure 1), associated sedimentation information is not part of their data schemes.

In general, reservoir bathymetry has not been surveyed systematically, and the survey information that may exist is not included in the National Reservoir Sedimentation Database (RESSED), which has no entries for Hawaii. Therefore, we are collecting, organizing, and analyzing existing physical data about reservoirs located on the main Hawaiian islands in order to achieve the following suite of research objectives:

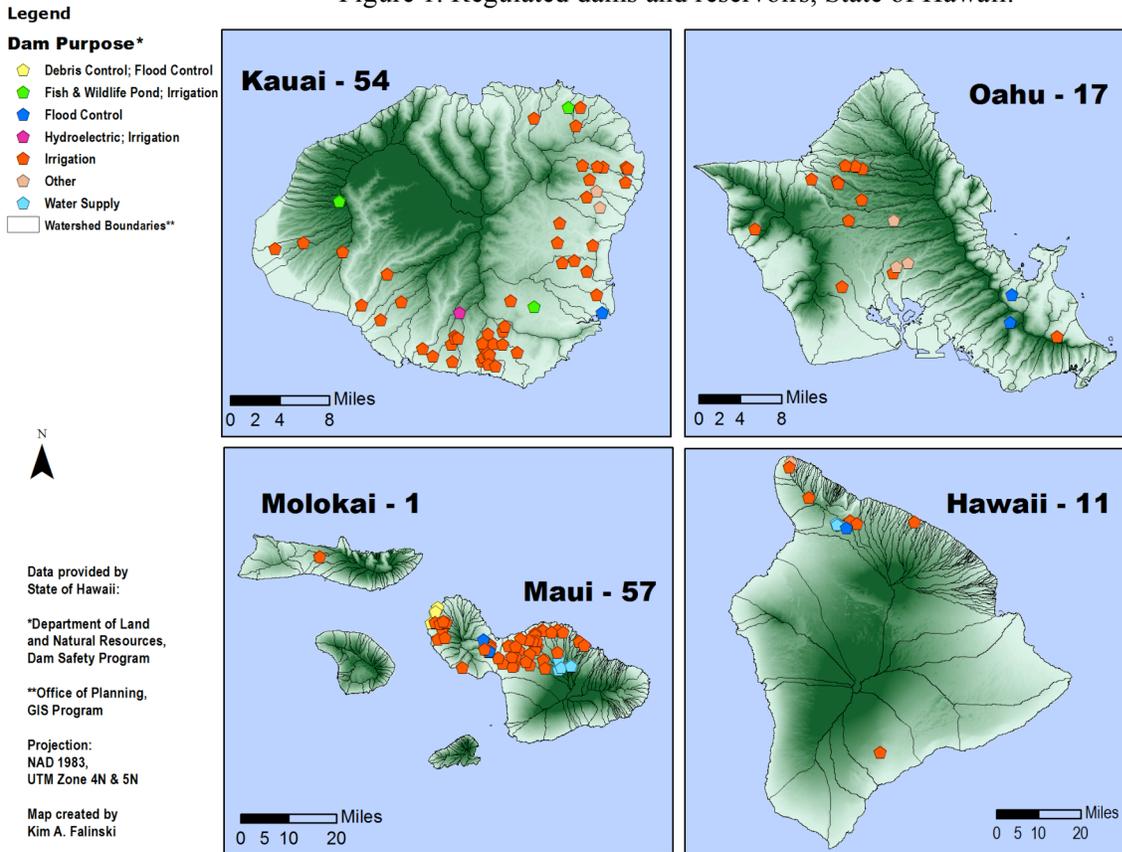
- (1) Obtain design and as-built bed elevations for all Hawaii reservoirs that are listed in the NID;
- (2) Obtain other reservoir design factors (drainage area sediment yield, source sediment and bed sediment bulk density, and reservoir storage capacity loss rate) for the same facilities;
- (3) Obtain corresponding data from subsequent bathymetric surveys, watershed analyses, sediment sampling, and hydrologic calculations;
- (4) Organize the collected information into a database that mimics the structure of RESSED;
- (5) Perform a gap analysis of the database records to identify remaining data needs;
- (6) Develop a sampling and analysis plan for a field and archival investigation to fill gaps in the historic record and establish new baselines for reservoir physical characteristics; and
- (7) Collaborate with other investigators, agencies, and interested parties to prepare and submit follow-on proposals for implementing the sampling and analysis plan and loading completed records to RESSED.

Methodology

Approximately 140 of the largest dams and reservoirs in the Hawaiian islands are regulated under state laws that govern dam and reservoir safety (see Dam Safety Program, 2009). The state program's digital inventory is the main source of Hawaii information for the NID (Civil Works Engineering Division, 2008) and provides the most accessible, detailed, and robust starting point

for obtaining, storing, and analyzing information about reservoir sedimentation. The state inventory holds 73 fields of information for each dam/reservoir record, including basic identifiers (name and id number); ownership and contact information; location (coordinates and parcel identifier); purpose; and hydrographic setting (watershed and stream).

Figure 1. Regulated dams and reservoirs, State of Hawaii.



The inventory includes other information that assists sedimentation analysis, such as dam height, reservoir size (surface area and storage volume), drainage area, and outlet and spillway design and discharge characteristics. However, storage volume values are based on design or as-built capacity to hold water and sediment; they do not reflect actual changes in water storage capacity caused by sedimentation, which is the information that RESSED is designed to hold.

The current RESSED structure includes 15 relational data tables and 375 data fields that users can manipulate to analyze sediment deposition. Most RESSED searches are linked by three data fields—reservoir identification number, survey date, and pool identification (Ackerman et al., 2009). Initial RESSED data collection and data entry was based on a standardized “Reservoir Sediment Data Summary” form developed by federal agencies (Steffan, 1996). Reservoir identification number is already provided by NID and the state inventory, and pool identification is only important for reservoirs with multiple pools, which rarely applies in Hawaii. Therefore, we are focusing on data collection to fill seven of the remaining 44 data fields found on the original standardized form:

- Field 26 (survey date)
- Volumetric survey results:

- Field 32 (storage capacity)
- Field 37 (period capacity loss)
- Field 38 (total sediment deposits to date)
- Field 41 (storage loss)
- Mass-based information:
 - Field 39 (soil bulk density)
 - Field 40 (tons of sediment deposited)

The data associated with these fields is not required or available in the NID and the state inventory; therefore the information must be acquired on a reservoir-by-reservoir basis from other sources. Other fields in RESSED that hold useful data for reservoir calculations and watershed analysis may be also populated as site-specific information is encountered, such as Fields 34 (period annual precipitation); 35 and 36 (water inflow by period and to date); and 42 (sediment inflow concentration).

We are searching a variety of sources to collect data on bathymetric surveys, reservoir design, and reservoir/watershed characteristics that can be used to fill these data fields, either directly or computationally. Sources include online geodatabases and data exchanges; published and unpublished technical studies, research papers, planning documents, and regulatory materials; and unpublished records held by individual dam owners, contractors, regulators, and technical/financial assistance agencies. The starting point for this effort is the reservoir-specific files maintained by the state dam safety program, supplemented by owner and agency records that we are accessing through a three-step outreach process: (1) send a letter of introduction to each of the forty main ownership entities identified in the state inventory; (2) make telephone contact; and (3) continue acquiring information accordingly, including office and site visits.

RESSED data for other states is currently accessible in several formats (Subcommittee on Sedimentation, 2013a). However, the timeframe for enabling public updating and retrieval from the forthcoming Filemaker Pro database management system is uncertain (Subcommittee on Sedimentation, 2013b). In light of this uncertainty, we chose to begin recording our data entries by adding the necessary fields to a Microsoft Excel version of the state inventory that we received from the state regulator, including fields that provide links to our project document archive. This provides a stand-alone platform for (a) gap analysis of the database records; (b) recording time and effort expended on project tasks; (c) cataloging resource materials and field methods; and (d) managing project information and developing plans and proposals for follow-up investigations. To promote interoperability with other national data systems and GIS hydrologic network applications, new RESSED data fields accommodate reach codes and measure values from the National Hydrography Dataset (NHD) that link with reservoir location; in our case, these geospatial attributes are available from the high-resolution (1:24,000 scale) Hawaii NHD (Penn and Kimura, 2009; data available for download at: <ftp://nhdftp.usgs.gov/DataSets/Staged/States/FileGDB/HighResolution>).

Principal Findings and Significance

The state inventory contains estimates of original reservoir storage capacity that provide a useful basis for analyzing the distribution of storage capacity among islands, owners, purposes, irrigation service areas, receiving waters, watershed contributing areas, and other significant

institutional and physical characteristics. For example, on a geographic basis, each of the three smaller islands of Kauai, Oahu, and Molokai has three to five times more reservoir storage, per unit area, than Maui, which has the greatest number of reservoirs (Table 1). Hawaii island—which covers an area larger than that of all the other islands combined—has only 3% of the total statewide storage capacity. Just 10% of all reservoirs account for 2/3 of total capacity, and the state’s two largest reservoirs—one on Kauai and one on Oahu—are privately owned and operated, and together account for 30% of total capacity. About 1/3 of all capacity is associated with reservoirs that are owned and operated by government agencies, primarily on Kauai (11 state/3 county) and Maui (1 state/12 county).

Table 1. Reservoir storage capacity ranked by storage per unit area.

Island	Normal Storage Capacity (acre-feet)	% of State Total	Number of Reservoirs	% of State Total	acre-feet per acre
TOTAL* (5 main islands)	36,210	<100	133	95	0.009
Kauai	15,553	43	52	37	0.044
Oahu	10,803	30	16	11	0.028
Molokai	4,365	12	1	1	0.026
Maui	4,347	12	54	39	0.009
Hawaii	1,242	3	10	7	<0.001

*The state inventory lacks normal storage capacity data for seven reservoirs out of 140 statewide.

However, these figures reflect optimum water storage conditions only, and do not account for decreases in storage capacity caused by reservoir sedimentation. Such changes are generally documented through recurring bathymetric surveys that quantify the rise in reservoir bed elevation over time. Our initial results indicate that resurveys are uncommon in Hawaii; reliable historic documentation of original reservoir bathymetry is sparse; and the bulk of the survey information that does exist is concentrated in the files of corporate and government offices located on Kauai and Maui.

Bed elevation is not routinely included in the data sources that we have reviewed, however knowing with certainty where the bottom of a reservoir is located is a repeated concern amongst owners and operators. In some cases, bed elevation may be roughly inferred from site topography and existing inventory data about dam height, dam base elevation, and reservoir depth (which itself must generally be derived from reservoir surface area and storage capacity). For about 60% of the reservoirs in the inventory, initial estimates of design bed elevation are available from 1970s-era investigations that include area-capacity curves, which indicate a base elevation at which storage capacity is zero (Harding-Lawson Associates, 1978). Therefore, during the coming project year we will continue compiling elevation data from these area-capacity curves, while also seeking data verification in other archival sources and assessing methods for field validation.

The availability of additional information about bed elevation and other reservoir design factors and physical characteristics varies widely. Our initial database queries, literature

searches, and personal contacts indicate that it would take considerable effort (beyond the resources of the current project) to acquire reasonably complete information about each of the 140 reservoirs in the state inventory. Therefore, we are first classifying the universe of reservoirs according to levels of information availability, then targeting a subset of reservoirs in each class for focused investigation, in order to estimate the degree of effort that might be required to complete data collection for all reservoirs.

Information availability is largely a function of ownership. Public works reservoirs and those owned by larger corporations tend to have the most complete information sets. However, private owners may choose to keep their records private. Based on our initial outreach results and background research, indicates the current status of our owner-based data collection plan (Table 2).

Table 2. Reservoir ownership and data collection status.

Owner Type*	Number of Reservoirs Per Owner	Number of Owners	Level of Participation and Status of Owner Data Collection
Private-single	1	15	Four owners are participating; three with recent/pending data collection
Private-multiple	2–7	12	One owner is participating; others pending
Private-major	13 and 21	2	Pending
Public-County Agency	1–6	6	Three owners engaged; all with recent/pending data collection
Public-State Agency	2–6	4	Three owners engaged; two with recent/pending data collection
Public-Federal	1	1	Pending (field visit scheduled)
Total		40	

*Many reservoirs have multiple owners; some private owners may be corporately interlocked with others. This table is based on the first owner listed in the state inventory and does not account for corporate interlocking.

Although water storage capacity curves exist for about 60% of the regulated reservoirs, information about actual sediment delivery, trapping efficiency, accumulated volume, and release is limited. Extreme spatial and temporal variability in watershed processes complicates the calculation of sediment delivery rates, and the unique geologic and climatic setting increases the uncertainty of using established formulas from other regions. However, many owners and operators have been performing new investigations to support dam removal and other compliance with revised dam safety regulations (Hawaii Administrative Rules Chapter 13-190.1, effective February 20, 2012, <http://hawaii.gov/dlnr/eng/rules>), and the public records of these investigations may provide useful information. For example, from July 2010–June 2012, ten dams were at some stage of the permitting and construction process for removal, with no pending enforcement actions against dam owners (Department of Land and Natural Resources, 2011, 2012). Although these investigations typically do not include measurement of reservoir bathymetry and determination of base elevation (and changes in bed elevation over time), there

are a few notable exceptions that serve as important examples for planning future work (AquaTechnex, LLC, 2009).

A more driving concern expressed by owners and operators—and one that may hold promise for future research—is to identify and employ effective and efficient sediment removal methods that meet environmental protection requirements. Our work with the reservoir community has also revealed that the financial aspect of reservoir sustainability is another critical, bottom-line concern for owners, operators, regulators, and taxpayers. In 2012, Hawaii voters rejected a proposed amendment to the state constitution that would have authorized the state to issue special purpose revenue bonds to assist dam and reservoir owners in complying with current safety standards (Engineering Division, 2012). The 2013 state legislature proposed a budget that includes line items for \$9 million of reservoir safety improvements in state-owned irrigation systems and \$1 million of improvements to one particular public reservoir (House of Representatives, 2013, enactment pending). A separate measure to provide \$2 million for improvements to a different public reservoir stalled during the current legislative session but could be started up again next year (The Senate, 2013). In the coming project year, we will continue to record financial statistics related to reservoir sustainability, laying groundwork for future research in developing related policy, regulations, programs, and services.

Through our consultations with the state Dam Safety Program and its regulated community, we learned about the existence of at least 300–400 unregulated dams that do not have reporting requirements and represent a significant gap in watershed management information. The smaller impoundments associated with these unregulated structures may represent a manageable and compelling nexus for watershed science research to complement our analysis of the larger, more complex systems associated with regulated dams (e.g., Verstraeten and Poesen, 2001, 2002). Therefore, it may be useful to pursue follow-up projects that would inventory physical data about these smaller impoundments by replicating the approach that we are following in this project.

Publications Cited in Synopsis

Ackerman, K.V., D.M. Mixon, E.T. Sundquist, R.F. Stallard, G.E. Schwarz, D.W. Stewart, 2009, RESIS–II: An updated Version of the original reservoir sedimentation survey information system (RESIS) database, U.S. Geological Survey Data Series 434, <http://pubs.usgs.gov/ds/ds434/ds434.pdf>.

AquaTechnex, LLC, 2009, Kalihiwai reservoir bathymetry study, http://www.kalihiwaireservoir.info/images/Kalihiwai_Reservoir_Bathymetry_Study_AquaTechnex.pdf

Civil Works Engineering Division (Headquarters, U.S. Army Corps of Engineers), Association of State Dam Safety Officials, and U.S. Army Topographic Engineering Center, 2008, National inventory of dams methodology, state and federal agency manual, version 4.0, http://www.damsafety.org/media/Documents/STATE_INFO/STATE_DATA_CALL/NID_MethodologyManual.pdf.

Dam Safety Program, Department of Land and Natural Resources, State of Hawaii, 2009, Program overview, <http://www.hidlnr.org/eng/dam/Overview.aspx>.

Department of Land and Natural Resources, State of Hawaii, 2011, Report on the Hawaii Dam Safety and Reservoir Program, State Fiscal Year 2011, report to the Twenty-Sixth

Legislature, Regular Session of 2012, <http://archive.jan2013.hawaii.gov/dlnr/reports-to-the-legislature/2012/EN12-Dam-Safety-Rpt-FY11.pdf>.

Department of Land and Natural Resources, State of Hawaii, 2012, The Hawaii Dam Safety and Reservoir Program, State Fiscal Year 2012, report to the Twenty-Seventh Legislature, Regular Session of 2013, <http://files.hawaii.gov/dlnr/reports-to-the-legislature/2013/EN13-Dam-Safety-Rpt-FY12.pdf>.

Engineering Division, Department of Land and Natural Resources, State of Hawaii, 2012, On the ballot, *Wai Halana, Hawaii Flood News* (October 2012): 1–2, <http://www.hidlnr.org/eng/nfip/WHpage9.aspx>.

Harding Lawson Associates; Department of the Army, Pacific Ocean Division, U.S. Army Corps of Engineers; 1978, Phase I Report: National Dam Safety Program, Wahikuli Dam, I.D. No. 55. Honolulu, Hawaii.

House of Representatives, Twenty-Seventh Legislature, State of Hawaii, 2013, HB200 HD1 SD1 CD1 (Relating to the State Budget), http://www.capitol.hawaii.gov/measure_indiv.aspx?billtype=HB&billnumber=200.

Penn, D., J. Kimura, 2009, Update, Enhancement, and Maintenance of the National Hydrography Dataset for Hawaii, in *Building Resilient Communities Conference*, Hawaii Congress of Planning Officials and Hawaii Geographic Information Coordinating Council, Honolulu, Hawaii, September 23–25.

Randle, T.J., K.L. Collins, J.R. Gray, 2013, Avoiding the inevitable? Capacity loss from reservoir sedimentation, *Eos*, 94(1): 4.

Steffan, L.J., 1996, A reservoir sedimentation survey information system—RESIS, in *Proceedings of the 6th Federal Interagency Sedimentation Conference*, March 25-29, 2001, Las Vegas, Nevada, I-29 to I-36, <http://ida.water.usgs.gov/ressed/references/steffen-1996.pdf>.

Subcommittee on Sedimentation, Advisory Committee on Water Information, U.S. Geological Survey, 2013a, The Reservoir Sedimentation Database (RESSED), Database Download and Documentation, http://ida.water.usgs.gov/ressed/db_doc2013.

Subcommittee on Sedimentation, Advisory Committee on Water Information, U.S. Geological Survey, 2013b, The Reservoir Sedimentation Database (RESSED), Welcome to the Reservoir Sedimentation (RESSED) Database, <http://ida.water.usgs.gov/ressed/index.cfm>.

The Senate, Twenty-Seventh Legislature, State of Hawaii, 2013, SB1362 (Relating to Capital Improvement Projects for the Benefit of the Fourth Senatorial District), http://www.capitol.hawaii.gov/measure_indiv.aspx?billtype=SB&billnumber=1362&year=2013.

Verstraeten, G., J. Poesen, 2001, The importance of sediment characteristics and trap efficiency in assessing sediment yield using retention ponds, *Physics and Chemistry of the Earth, Part B: Hydrology Oceans and Atmosphere*, 26(1): 83–87.

Verstraeten, G., J. Poesen, 2002, Using sediment deposits in small ponds to quantify sediment yield from small catchments: possibilities and limitations, *Earth Surface Processes and Landforms*, 27(13): 1425–1439.

Usage of UV disinfection coupled with rain water catchment and stream water in rural areas

Basic Information

Title:	Usage of UV disinfection coupled with rain water catchment and stream water in rural areas
Project Number:	2012HI377B
Start Date:	3/1/2012
End Date:	2/28/2013
Funding Source:	104B
Congressional District:	Hawaii 1st
Research Category:	Engineering
Focus Category:	Water Supply, Water Quality, Water Use
Descriptors:	
Principal Investigators:	Bunnie Yoneyama, Joseph J. Lichwa

Publications

There are no publications.

FINAL REPORT

**Usage of UV Disinfection Coupled with Rain Water
Catchment and Stream Water in Rural Areas**

May 2013

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Matteo D'Alessio

Project Number: 2012HI377B

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Introduction

Water is a necessity for human life. In many developing countries or in the aftermath of a disaster, the amount of clean water available for use and consumption can be severely limited. One inexpensive, relatively simple means of improving water quality is slow sand filtration (SSF). From our own experience and from many other studies [1,2,3] it has been shown that slow sand filtration can remove approximately 90–99.9% of total coliform and *E. coli* from the input water. If the quality of the feed water, however, is poor, total coliform and *E. coli* may still be present in the output from the SSF.

There are a number of point of use methods that can be used after SSF to further improve water quality. In keeping with what is available in third world countries or disaster hit areas, the method should be inexpensive, reliable, easy to use and easy to maintain. One simple method is addition of a disinfectant chemical such as sodium hypochlorite to the sand filtered water. The cost is low, however, there is some complexity to calculating the proper dose of chemical. If the dosed amount is too low, adequate disinfection may not be achieved. If, on the other hand, the dose is too high, the unpleasant taste and smell may discourage use of the water.[4] With the use of chlorine, there is also the problem of the formation of disinfection by products some of which are considered carcinogenic.[5,6]

A better choice may be a UV disinfection unit, which can be as simple as a tube containing a germicidal lamp. The disinfection occurs when the input water is exposed to the UV irradiation produced by the lamp. Dosing is taken care of primarily in the construction of the unit and UV disinfection produces no known disinfection by products.[7]

A commercial UV unit able to handle flows of 500 ml/min can be as inexpensive as \$100 but may not be readily available at that price everywhere in the world. It is possible to make a UV unit for half that cost, but more importantly aside from the germicidal lamp, it can be made with locally available materials. A UV unit was made by following a plan presented in a Master's project paper entitled "The UV-Tube as an Appropriate Water Disinfection Technology: An Assessment of Technical Performance and Potential for Dissemination" by Alicia Cohn.

The UV unit in the paper was designed so that the germicidal bulb is hung over the input water. The water is exposed to UV irradiation as it travels from the input to the output. It is relatively easy and inexpensive to purchase a 15W 18" germicidal lamp. It is much harder and more expensive to find a germicidal lamp that can be submersed in the sample. Having a bulb that is hung above the input water also prevents the problem of lamp fouling by the input water.

A 15W 18" germicidal lamp, was attached to the top of a 4" diameter 20" long PVC pipe. Because ultraviolet radiation is known to degrade plastic, the PVC pipe was lined with galvanized steel sheeting used for roofing. Stainless steel is also generally accepted as a safe material to use with UV light but galvanized steel is less expensive and readily available because of its use for gutters and flashing. The galvanized steel sheet was epoxyed to the pipe so that no water would be caught between the sheet and the pipe. Plastic pipe end caps were used to seal the ends of the PVC pipe. Tubing was used to deliver water into and out of the pipe. The height of the exit tubing inside the pipe determined the maximum depth of the input water and the maximum flow rate that could be achieved. The homemade UV unit cost approximately \$45.

After assembly, flow was tested through the unit using both gravity and a pump. It was able to achieve a maximum flow rate of 300 ml/min.

Mechanism of Disinfection

UV light is generally defined as any wavelength of electromagnetic radiation shorter than 400 nm. UVA comprises wavelengths from 315–400 nm; UVB from 280–315 nm; and UVC from 200–280 nm.

Ultraviolet light adsorption by bacteria is due chiefly to purine and pyrimidines of nucleic acids with maximum adsorption occurring at 260–265 nm (UVC). The adsorption of UVC causes alterations in the DNA which block replication. There is, however, a potential for the bacterial cells to repair themselves either in the dark or by exposure to visible and near UV light.[8]

Dose

Dose is the product of irradiance and exposure time. Irradiance is a function of the bulb and it decreases with distance from the bulb. The geometry of the UV unit and the composition of the input water will determine how quickly the irradiance decreases with distance from the bulb. Exposure time of the sample is governed by the geometry of the UV unit.[9]

The performance of the UV unit is also affected by the quality of the input water. UV light is adsorbed by certain substances in the water including natural organic matter (NOM), nitrate, iron, and manganese. As the amount of these materials in the water increases, absorbance of UV light by them increases and transmittance decreases.[10]

We did not choose to design a system such that the lowest dose received by any of the input water is sufficient theoretically to achieve a certain amount of disinfection. Instead we chose to follow the simplest plan and then evaluate performance of the system on an environmental sample.

Methodology

Input water was fed through the homemade UV unit using a Masterflex peristaltic pump at a specific flow rate. Total coliform and *E. coli* or fecal coliform were monitored before and after UV treatment. To give a better idea of overall performance, sample collection following UV treatment occurred over a period of 20–30 minutes. Total sample collected was 1L. The homemade unit was constructed so that power could be supplied either by direct current—the unit was plugged into an electrical outlet—or by using a solar panel. This allowed a performance evaluation of the UV unit itself without the added variable of power supply. For comparison, input water was also treated by a commercial UV unit, SunPure Model UST-200EB.

The amount of total coliform and *E. coli* were initially assayed using IDEXX, a commercial MPN system. However, part way through the study, difficulty reading the results of samples containing sewage were encountered which forced a switch to membrane filtration. Percent reduction of a microorganism after treatment was calculated using the formula $(1 - (C_i/C_o)) * 100$.

IDEXX is a commercial most probable number system that uses a defined substrate technology to measure both total coliform and *E. coli* simultaneously. If total coliform are present in the sample, the B-galactosidase present in the total coliform act on ONPG in the

IDEXX reagent, which is called Colilert 18 and turn the sample yellow. If *E. coli* are present in the sample, they contain B-glucuronidase which acts on the MUG in the Colilert 18 and turns the sample fluorescent blue under long wave UV light. The results are reported as most probably number (MPN)/100 ml. Colilert 18 is EPA approved and is included in Standard Methods for the Examination of Water and Wastewater.[11]

Membrane filtration consists of filtering a sample through a 0.45 μm filter. Anything larger than 0.45 μm , which includes the bacteria, remain on the top of the membrane filter. The filter is then placed on a specific agar medium containing not only necessary nutrients but often selective and differential reagents which help separate the bacteria of interest from the rest. For total coliform the medium used was mEndo-LES and for fecal coliform the medium was mFC agar. Each positive colony is assumed to be the product of one bacteria. Colonies are counted; adjusted for inoculum size and any dilution and reported as colony forming units (CFU)/100 ml. Membrane filtration is EPA approved and included in Standard methods for the Examination of Water and Wastewater.[12]

The input water was Manoa Stream or Manoa Stream mixed in various ratios with primary effluent from Honouliuli Wastewater Treatment Plant. The stream and wastewater mix contained more bacteria and more natural organic matter than the stream alone and therefore was a more challenging sample.

A variety of flow rates were used ranging from 55 ml/min to 300 ml/min. The lower the flow rate, the longer the sample is exposed to the UV light which should lead to increased disinfection of the sample. All the flow rates are within the limits of production by a slow sand filter.

The solar panel was a 20 watt Siemens model SM20 solar panel. Total solar irradiance was recorded during the experiments but was unhelpful in predicting when the solar panel would generate enough power to keep the germicidal lamp on. It was easier to monitor when the germicidal lamp in the unit was “on”. The lamp should not be viewed directly with unprotected eyes, but the blue light of the lamp could be seen on the exit tubing.

Results

- 1) The homemade UV unit was first evaluated to see if its performance was comparable to a commercial unit. Both the homemade and commercial unit were run with Manoa Stream water or Manoa Stream spiked with wastewater (WW) using direct current as the power source.
 - Tables 1 and 2 show that at all flow rates and even with starting bacterial numbers in the 100,000 range, the homemade unit performed as well as the commercial unit.
 - Table 3 shows the results of the same kind of experiment but with even higher starting numbers of total coliform and *E. coli*. Unfortunately the only assays that could be read accurately were those done at the highest flow rates. Because disinfection is usually better at lower flow rates (longer exposure time of the sample to UV), the results at 55 ml/min and 170 ml/min were probably the same or better than that at 285 ml/min.
- 2) The homemade unit was then tested using the solar panel as the source of power. The homemade unit was also run using direct current. First tests were done on the lawn in front of Holmes Hall.

- In Table 4 the stream water was assayed with IDEXX, and the stream water spiked with sewage was assayed using both IDEXX and membrane filtration. Because of the difficulty reading the IDEXX results on the samples with sewage, the membrane filtration results were used. In all later experiments, total coliform and fecal coliform are assayed by membrane filtration.

The homemade unit powered by the solar panel reduced total coliform by 98–99.9% and *E. coli* or fecal coliform by 98–100%. Lower flow rates result in better disinfection than the high flow rates. With identical flow rates, when the homemade unit is run by direct current, there is greater reduction in both microorganisms than when the unit is run by the solar panel. It may be that any slight shadow on the solar panel, a cloud or bird for example, causes the power to drop and the germicidal lamp is momentarily off leaving some sample untreated.

- Table 5 is another example of the results when using the homemade unit powered by the solar panel. Although there is little or no reduction in either bacteria in the first three samples, samples done a few hours later on the same day show at least 90% reduction in total coliform and at least 88% reduction in fecal coliform. It was observed during the sample runs that the unit turns off frequently—the solar panel is not able to provide consistent adequate power to keep the germicidal lamp on. The last sample was processed using the UV unit powered by direct current. The resulting 96% reduction in total coliform and 99.8% reduction in fecal coliform can probably be considered the “best” the homemade unit can achieve on this particular sample.

Again, better disinfection is obtained with the lower flow rate.

- Table 6 shows the results of the experiment done on the roof of Hawaii Institute of Geophysics building. An attempt was made to use the solar panel under optimal conditions—a very hot, very sunny, cloudless day.

The homemade unit with solar power performed as well as the handmade unit with direct current. With solar power the reduction in total coliform is 98.48% and 99.95%, while with direct current, the reduction is 99.96%. The same is true for fecal coliform.

The stream spiked with sewage was also run through a commercial unit powered by direct current. The reduction in total and fecal coliform was more than with the handmade unit powered by direct current. This may be because, in the commercial unit, the sample flows completely around the germicidal lamp rather than flowing below the lamp as in the homemade unit.

Conclusions and Recommendations

The homemade UV unit was cheap and fairly easy to make. It did not require any sophisticated equipment.

The homemade UV unit performance was comparable to the commercial UV unit.

The solar panel was not able to provide consistent and adequate power to the homemade UV unit. Instead of looking for a bigger or better performing solar panel, we would recommend looking for a germicidal lamp with a lower wattage. Ideally, it would be a submersible germicidal lamp.

References Cited

1. Huisman, L., and W.E. Wood, 1974, Slow sand filtration, World Health Organization.
2. Weber-Shirk, M.L., and R.I. Dick, 1997, Biological mechanisms in slow sand filters, *Journal of American Water Works Association*, 89(2), 72–83.
3. Hijen, W.A.M., J.F. Schijran, P. Bonne, A. Visser, G.J. Medema, 2004, Elimination of viruses, bacteria and protozoan cysts by slow sand filtration, *Water Science and Technology* 50(1), 147–154.
4. Burch, J., and K.E. Thomas, 1998, An overview of water disinfection in developing countries and the potential for solar thermal water pasteurization. Golden, Colorado, National Renewable Energy Laboratory.
5. Rook, J.J., 1974, Formation of haloforms during chlorination of natural waters, *Water Treatment Examination*, 23, 234–243.
6. Disinfection by products and the safe water system, 2013, http://www.cdc.gov/safewater/publications_pages/thm.pdf.
7. Wastewater Engineering Treatment and Reuse, 4th edition, 2003, Metcalf and Eddy, revised by G. Tchobanoglous, F.L. Burton, H.D. Stensel, p. 1332.
8. Microbiology, 3rd edition, 1980, B.D. Davis, R. Dulbecco, R. Eisen, H.N. Ginsberg, eds. 186–189.
9. Cohn, A., 2002, The UV-tube as an appropriate water disinfection technology: an assessment of technical performance and potential for dissemination, Energy and Resources Group, University of CA, Berkeley, p. 13.
10. Wastewater Engineering Treatment and Reuse, 4th edition, 2003, Metcalf and Eddy, revised by G. Tchobanoglous, F.L. Burton, H.D. Stensel, p. 1308.
11. http://www.idexx.com/view/xhtml/en_us/water/products/cololert-18.jsf
12. Standard Methods for the Examination of Water and Wastewater, 21st edition, 2005, Edited by A.D. Eaton, L.S. Clesceri, E. Rice, A.E. Greensberg, American Public Health Association, Washington, D.C., 20001–3710, 9-59 to 9-68.

Table 1. Percent reduction of total coliform and *E. coli* in samples after treatment with the homemade UV unit and a commercial UV unit.

Sample/Treatment	Flow Rate (ml/min)	Total Coliform		<i>E. coli</i>	
		(MPN/100 ml)	% Reduction*	(MPN/100 ml)	% Reduction*
Manoa Stream		1.58E+04		1.31E+03	
Stream/Homemade UV ^a	55	<1	100%	<1	100%
Stream/Commercial UV ^b	55	<1	100%	<1	100%
Stream/Homemade UV	170	<1	100%	<1	100%
Stream/Commercial	170	1	100%	1	100%
Stream/Homemade	285	<1	100%	<1	100%
Stream/Commercial	285	1	100%	<1	100%

* % Reduction = $(1 - (C_i/C_0)) * 100$.

^aStream/Homemade UV = stream water treated by the homemade UV unit.

^bStream/Commercial = stream water treated by a SunPure Model UST 200 EB UV unit.

Table 2. Percent reduction of total coliform and *E. coli* in samples spiked with wastewater after treatment with the homemade UV unit and a commercial UV unit.

Sample/Treatment	Flow Rate (ml/min)	Total Coliform		<i>E. coli</i>	
		(MPN/100 ml)	% Reduction*	(MPN/100 ml)	% Reduction*
Manoa Stream+WW (10:1) ^a		8.29E+05		3.36E+05	
Stream+WW/Homemade ^b	55	<1	100%	<1	100%
Stream+WW/Commercial ^c	55	<1	100%	<1	100%
Stream+WW/Homemade	170	<1	100%	<1	100%
Stream+WW/Commercial	170	1	100%	<1	100%
Stream+WW/Homemade	285	1	100%	1	100%
Stream+WW/Commercial	285	1	100%	<1	100%

* % Reduction = $(1 - (C_i/C_0)) * 100$.

^aStream+WW (10:1) = the sample is stream water mixed with wastewater in a ratio of 10:1.

^bStream+WW/Homemade = stream water mixed with wastewater is treated by the homemade UV unit.

^cStream+WW/Commercial = stream water mixed with wastewater is treated by a SunPure UV unit.

Table 3. Percent reduction of total coliform and *E. coli* in wastewater spiked samples after treatment with the homemade UV unit and a commercial UV unit.

Sample/Treatment	Flow Rate (ml/min)	Total Coliform		<i>E. coli</i>	
		(MPN/100 ml)	% Reduction*	(MPN/100 ml)	% Reduction*
Manoa Stream+WW (3:1) ^a		6.97E+07		1.61E+07	
Stream+WW/Homemade ^b	285	<10	99.999%	<10	99.999%
Stream+WW/Commercial ^c	285	<10	99.999%	<10	99.999%

* % Reduction = $(1 - (C_i/C_0)) * 100$.

^aManoa Stream+WW (3:1) = the sample is stream water mixed with wastewater in a ratio of 3:1.

^bStream+WW/Homemade = stream water mixed with wastewater is treated by the homemade UV unit.

^cStream+WW/Commercial = stream water mixed with wastewater is treated by the SunPure UV unit.

Table 4. Percent reduction of total coliforms and *E. coli* or fecal coliforms after treatment by the homemade UV unit when powered by a solar panel.

Sample/Treatment	Flow Rate (ml/min)	Total Coliform		<i>E. coli</i>	
		(MPN/100 ml)	% Reduction*	(MPN/100 ml)	% Reduction*
Manoa Stream		1.83E+04		2.40E+02	
Stream/UV Sun ^a	300	1.09E+02	99.404%	4.1	98.292%

Sample/Treatment	Flow Rate (ml/min)	Total Coliform		Fecal Coliform	
		(MPN/100 ml)	% Reduction*	(CFU/100 ml)	% Reduction*
Stream+WW ^b		7.00E+06		3.44E+06	
Stream+WW/UV Sun ^c	100	1.12E+03	99.984%	0	100%
Stream+WW/UV Sun	300	8.76E+04	98.749%	1.68E+04	99.512%
Stream+WW/UV Electricity ^d	300	3.72E+03	99.947%	16	98.999%

Note: The stream water samples were analyzed with the IDEXX system. Because of a technical difficulty, the stream and wastewater mix was analyzed using membrane filtration.

* % Reduction = $(1 - (C_i/C_0)) * 100$.

^aStream/UV Sun = stream water is treated by the handmade UV unit which is powered by a solar panel.

^bStream+WW = the sample is stream water mixed with wastewater in a ratio of 5:1.

^cStream+WW/UV Sun = stream water spiked with wastewater is treated by the homemade UV unit which is powered by a solar panel.

^dStream+WW/UV Electricity = stream water mixed with wastewater is treated by the homemade UV unit which is powered by direct current.

Table 5. Percent reduction of total coliforms and fecal coliform in samples after treatment by the homemade UV unit when powered by a solar panel.

Sample/Treatment	Flow Rate (ml/min)	Total Coliform		Fecal Coliform	
		(MPN/100 ml)	% Reduction*	(CFU/100 ml)	% Reduction*
Manoa Stream		3.72E+04		3.08E+03	
Stream/UV Sun ^a	100	3.92E+04	no reduction	4.40E+02	85.714%
Stream/UV Sun	300	4.04E+04	no reduction	3.63E+03	no reduction
Stream+WW ^b		1.28E+07		2.56E+06	
Stream+WW/UV Sun ^c	100	5.56E+05	95.656%	5.48E+03	99.786%
Stream+WW/UV Sun (A1)	300	1.18E+06	90.781%	3.00E+05	88.281%
Stream+WW/UV Sun (A2)	300	4.84E+05	96.219%	4.12E+03	99.839%

* % Reduction = $(1 - (C_i/C_0)) * 100$.

^aStream/UV Sun = stream water is treated by the homemade UV unit which is powered by a solar panel.

^bStream+WW = stream water is mixed with wastewater in a ratio of 5:1.

^cStream+WW/UV Sun = stream water mixed with wastewater is treated by the homemade UV unit which is powered by a solar panel.

Table 6. Percent reduction of total coliforms and fecal coliform in samples after treatment by the homemade UV unit powered by a solar panel under optimal solar conditions.

Sample/Treatment	Flow Rate (ml/min)	Total Coliform		Fecal Coliform	
		(MPN/100 ml)	% Reduction*	(CFU/100 ml)	% Reduction*
Manoa Stream		1.60E+04		2.60E+02	
Stream/UV Sun ^a	100	20	99.875%	12	95.385%
Stream/UV Sun (A1)	300	44	99.725%	0	100%
Stream/UV Sun (A2)	300	28	99.825%	0	100%
Stream/UV Electricity ^b	300	28	99.825%	0	100%
Stream+WW ^c		5.80E+06		1.80E+06	
Stream+WW/UV Sun (B1) ^d	100	7.88E+02	99.986%	1.32E+02	99.993%
Stream+WW/UV Sun (B2)	100	3.08E+02	99.994%	80	99.996%
Stream+WW/UV Sun (C1)	300	8.80E+04	98.483%	7.96E+02	99.956%
Stream+WW/UV Sun (C2)	300	2.80E+03	99.952%	6.64E+02	99.963%
Stream+WW/UV Electricity ^e	300	2.28E+03	99.961%	7.64E+02	99.958%
Stream+WW/Commercial Electricity ^f	300	84	99.998%	6	99.999%

*% Reduction = $(1 - (C_i/C_0)) * 100$.

^aStream/UV Sun = stream water is treated by the homemade UV unit which is powered by a solar panel.

^bStream/UV Electricity = stream water is treated by the homemade UV unit powered by a direct current.

^cStream+WW = stream water is mixed with wastewater in a ratio of 5:1.

^dStream+WW/UV Sun = stream water mixed with wastewater is treated by the homemade UV unit which is powered by a solar panel.

^eStream+WW/UV Electricity = stream water mixed with wastewater is treated by the homemade UV unit which is powered by direct current.

^fStream+WW/Commercial Electricity = stream water mixed with wastewater is treated by the SunPure UV unit which is powered by direct current.

Bioaccumulation and Biotransformation of Arsenic by Marine Algae in Hawaii

Basic Information

Title:	Bioaccumulation and Biotransformation of Arsenic by Marine Algae in Hawaii
Project Number:	2012HI389B
Start Date:	3/1/2012
End Date:	2/28/2014
Funding Source:	104B
Congressional District:	Hawaii 1st
Research Category:	Water Quality
Focus Category:	Water Quality, Surface Water, Toxic Substances
Descriptors:	
Principal Investigators:	Philip Moravcik

Publications

There are no publications.

Problem and Research Objectives

For several decades arsenical compounds were used in the Hawaiian Islands as pesticides in the sugar industry. Although their use was basically discontinued in the 1940s much arsenic remains in the soil of former sugar cane fields and has been continually transported mostly bound to soil particles with water into the coastal waters of the state.

Seaweeds, or *limu* in Hawaiian, are an important part of Hawaiian cuisine and several species are highly prized by local cooks. It is common practice in Hawaii to gather these algae along the shorelines where they wash up.

Arsenic is famously toxic in several of its forms and therefore there are justifiable concerns about the safety of consuming algae from waters subject to arsenic contamination. This concern is reinforced by the fact that some seaweeds are known to concentrate arsenic (Diaz et al., 2011, Granchinho et al., 2001).

Recent research conducted on behalf of The Nature Conservancy in the State has revealed that seaweed collected at certain shoreline areas in the islands contains relatively high concentrations of arsenic.

Algae can transform arsenic between a number of states, metabolizing the arsenate and arsenite to less toxic methylated forms (Granchinho et al., 2001). This has implications for the risk posed by consumption of algae and its use as a soil amendment.

The objective of this study is to measure the arsenic content in algae collected from nearshore waters around the island of Oahu and to characterize the speciation of any arsenic to see what form it is occurring in.

Methodology

We collected preliminary samples of an alga believed to be *Gracilaria salicornia* (Figure 1) from the shore at Waikiki beach in Honolulu (21.265253°N, -157.822206°W, Figure 2). *Gracilaria salicornia* is one of the most successful invasive algae on reef flats in Hawaii. It is related to, and competitive with, the popular edible alga *Gracilaria coronipfolia*. The collected samples were freeze dried the day after sampling and the freeze dried material sent to our collaborating laboratory at the Illinois Sustainable Technology Center (ISTC) at the University of Illinois at Urbana-Champaign for analysis. Preliminary analyses were not able to speciate fully the total arsenic found in the sample. Subsequent work by John W Scott, ISTC Senior Analytical Chemist, and his team resulted in a fuller accounting of the different species that made up the total arsenic found in the sample.



Figure 1: *Gracilaria salicornia*



Figure 2: Sample collection site

Sample Preparation

The sample was homogenized with the aid of a gyromill and was milled to a fine powder (Figure 2).



Figure 3: Milled *G. salicornia* sample

Total Arsenic Digestion: To prepare the sample for total arsenic analysis by inductively coupled plasma mass spectrometry (ICP-MS), a microwave digestion procedure was employed. A quarter gram of homogenized sample was digested in a CEM microwave digestion system for 40 minutes with the aid of 10 ml nitric acid and 1 ml hydrogen peroxide. After cooling the sample was transferred to a centrifuge tube and diluted to a final volume of 50 ml. In addition, a reagent blank, an arsenic standard matrix spike, and a certified dogfish muscle tissue sample were processed in parallel to the sample to verify sample preparations. A matrix effect was observed for these samples; therefore a second microwave procedure was performed (See Total Arsenic Analysis Section). The second microwave digestion employed was identical to the first with the exception that one tenth of a gram homogenized sample was processed. In addition, the trifluoroacetic acid (TFA) extracted solids (0.078 g) for one algae sample was digested in this batch as well.

Extraction Methods: To prepare the algae sample for arsenic speciation analysis by liquid chromatography inductively coupled plasma mass spectrometry (LC-ICP-MS), solid-liquid extractions were performed. A quarter gram of homogenized sample was treated with 5.0 ml TFA at 65°C for two hours. Afterwards, the sample was shaken on a laboratory mixer for fifteen minutes. The TFA was collected and the procedure was repeated two more times while the TFA phases were pooled. The pooled fraction was then centrifuged for twenty minutes at 2000 RPM and the final TFA fraction was decanted into a drying tube. The TFA was then removed under a gentle stream of nitrogen and the residue was reconstituted in 10 ml 0.2% hydrochloric acid. A reagent blank, an inorganic arsenic matrix spike, and a certified dogfish muscle sample were processed in parallel to verify sample extraction.

Total arsenic analysis of the TFA extracts indicated that the extraction of arsenic was incomplete; therefore a second extraction of one of the TFA extracted solids was performed. The second extraction was identical to that of the first with the exception that a methanol-water (3:1) extraction fluid was utilized and the extraction was performed at 55°C. A reagent blank, an inorganic arsenic matrix spike, and a certified dogfish muscle sample were processed in parallel to verify sample extraction.

Total Arsenic Analysis: Total arsenic analysis was performed with a VG Elemental PQ Excel ICP-MS. Yttrium was utilized as an internal standard and the instrument was calibrated daily with reference materials procured from SPEX Certiprep. Verification of instrument calibration was achieved with preparations and analysis of two independent reference materials from the same vendor, but with different lot numbers. These check standards were analyzed post calibration and post sample analysis. Each ICP-MS measurement was conducted in triplicate and a sample duplicate and an analytical sample spike was performed during each assay.

Arsenic Speciation Analysis: Arsenic speciation was achieved with a liquid chromatography system interfaced to the ICP-MS instrument operated in a transient acquisition mode. Separation of the arsenic compounds was achieved with a Phenomenex Luna C18 100A column (250 x 4.40 x 5 μ) with a isocratic mobile phase of 2.5 mM oxalic acid, 10mM 1-heptanesulfonic acid (ion-pairing agent) and 0.1% methanol adjusted to a pH of 4 with ammonium hydroxide. The mobile phase flow rate was set at 1.0 ml/min and an injection volume of 30 μ l was used. Yttrium prepared at a concentration of 150 ng/ml in mobile phase was employed as an internal standard. Injection of 10 μ l of the internal standard was performed post-column and is necessary since mobile phase and sample salts dampen the signal intensity over the course of the assay. Calibration of the instrument was conducted with reference materials obtained from SPEX Certiprep, Sigma, and Chem Service. Verification of instrument calibration was achieved with preparations of reference materials by another chemist other than the one who prepared the calibration standards. Check standards were analyzed post calibration and post sample analysis.

Principal Findings and Significance

Total Arsenic Results: Table 1 presents the final results for total arsenic in digested samples and extraction samples. A significant matrix effect was observed during measurements of total arsenic in digestion batch one. This was indicated by low recoveries of the digestion matrix spike, digestion certified reference material (SRM), and the analytical spike. Therefore, a method of standard addition was performed on one sample, the matrix spike, and the SRM. Digestion quality controls were much improved under these conditions and confidence in the final arsenic result was obtained. When a smaller digestion mass was utilized in digestion batch 2, the matrix effect was not observed and method of standard addition was not necessary.

Table 1: Total Arsenic Results for Digested and Extracted Algae Samples

	Arsenic, mg/g	Digestion / Extraction Duplicates %RSD/%RPD	Matrix Spike / Reagent Blank Spike, %Recovery	Dogfish Muscle (DORM-2) SRM, %Recovery
Digestion Batch 1	13*	5.3%	60%* / NA	90%
Digestion Batch 2	10.2	17%	83% / 87%	88%
Extraction 1 (TFA)	5.9	8.0%	NA / 83%	92%
Digestion Batch 2 (TFA Solids)	4.7	NA	NA	NA
Extraction 2 (MeOH-Water)	2.6	NA	NA / 59%	87%

* - Determined by Method of Standard Addition

NA - Parameter Not Available

Total arsenic analysis of the TFA extract indicated that 51% of the total arsenic is extracted under these conditions. This was further verified by digestion and analysis of the extraction raffinate to account for 41% of the missing arsenic. Analysis of the second extraction (Methanol-Water 3:1), accounted for 22% of the unextracted arsenic from the first extraction. By summing the percent arsenic extracted by method one and method two, a total arsenic extraction of 73% was achieved.

Arsenic Speciation Results: Table 2 presents the final results for arsenic speciation of the algae extracts. The TFA extraction blank showed significant arsenate signal with regards to arsenate signals observed for samples. Most likely this is due to a argon-chloride interference (Ar40Cl35 at As75). This is also the most likely culprit for the high SRM recoveries for this species as well. Please refer to the discussion section of this progress report for a more details. The methanol-water extraction reagent blank spike recovered low for arsenite, however this was also seen in a low recovery for total arsenic in this sample as well. The most likely cause for this low recovery is that the sample was inappropriately spiked with arsenite.

Known arsenic species measured in this experiment were very low and do not account for the majority of arsenic species present in the algae sample.

Table 2: Arsenic Speciation Results for Algae Extracts (Concentration Units mg/g Unless Otherwise Noted)

	Arsenate (As ⁺⁵) as Arsenic	Arsenite (As ⁺³) as Arsenic	Monomethylarsinic acid (MMA) as Arsenic	Dimethylarsinic acid (DMA) as Arsenic	Arsenobetaine as Arsenic
TFA Extraction Blank	0.21	< 0.1	< 0.1	< 0.1	< 0.1
TFA Algae Extract 1	0.46	0.56	< 0.1	0.11	< 0.1
TFA Algae Extract 2	0.32	0.49	< 0.1	< 0.1	< 0.1
TFA Reagent Blank Spike	105% Recovery	84% Recovery	NA	NA	NA
TFA DROM-2 SRM Extract	323% Recovery	< 0.1	< 0.1	79% Recovery	2% Recovery
MeOH-Water Extraction Blank	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
MeOH-Water Algae Extract 1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
MeOH-Water Algae Extract 2	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
MeOH-Water Reagent Blank Spike	117% Recovery	12% Recovery	NA	NA	NA
MeOH-Water DROM-2 SRM Extract	198% Recovery	< 0.1	< 0.1	85% Recovery	97% Recovery

Discussion: Data obtained in this experiment are inconclusive if TFA is the best solvent for extraction of arsenic species in algae. This solvent extracted only 51% of the total arsenic contained in the algae sample. In addition, recoveries of arsenobetaine in the SRM were extremely low and reagent blanks for arsenate in this solvent were significant with regards to sample. Smith et al. (2008) reported success with TFA extraction of rice plants, however there is no mention of extraction blanks, no mention of extracted SRMs, and the research was only concerned with arsenate, arsenite, monomethylarsinic acid (MMA), and dimethylarsinic acid (DMA) (1). The use of a chloride interference correction may remedy the issue (see below discussion on interference corrections), however this solution has yet been demonstrated. Kohlmeyer et al. (2002) have reported that marine algae lack arsenobetaine and contain mostly arsenosugars (2). Given that, the effect of TFA may be moot point, however there is still concern that TFA will affect arsenosugars and one wonders, what is happening to the arsenobetaine? Increasing the current arsenic speciation data acquisition time and re-analyzing the TFA extracts to look for later eluting arsenic compounds may be an experiment worth conducting. In addition, setting up the current LC-ICPMS system to run arsenosugars would also be a direction worth heading. However, obtaining arsenosugar reference materials may prove futile (see below discussion on reference materials).

Methanol-water (3:1) extracted arsenic species that were un-extractable with TFA. The reagent blank indicated that the reagents used to prepare the extraction fluids produce less of interference. This result was further demonstrated by a lower recovery of arsenate in the SRM extracted by methanol-water, than that obtained for TFA (198% versus 323%). Arsenobetaine recoveries using the methanol-water extraction procedure were much superior to the TFA extracted counterpart and the DMA extraction recovery was greater as well. Increasing the current arsenic speciation data acquisition and re-analyzing the methanol-water extracts to look for later eluting arsenic compounds may be an experiment worth conducting. Also, setting up the current LC-ICPMS system to run arsenosugars would be a direction worth pursuing. An initial extraction of the algae material with methanol-water (3:1) is planned and results from this experiment are eagerly anticipated.

Observation of the argon chloride interference warrants concern. Many times this can be corrected by subtraction of the reagent blank, however since chloride is anticipated to be variable in samples this practice is unacceptable. Another approach to this issue is to utilize an interference correction equation. Arsenic is monoisotopic, with an atomic mass of 75 Daltons. Chloride has two isotopes, 35 Daltons and 37 Daltons, with relative abundances 75.53 and 24.47 respectively. If one monitors the signal at mass 77 Daltons (Ar⁴⁰Cl³⁷), then an interference equation can be employed to correct for the interference. However, one must still beware because selenium also has an isotope at 77 Daltons with a relative abundance of 7.58. Therefore, monitoring selenium at mass 82 would allow one to correct for selenium interference at the Ar⁴⁰Cl³⁷ mass. Still yet we are not out of the woods, krypton has an isotope at mass 82 as well, with a relative abundance of 11.56. Krypton is typically found alongside argon and because it is heavier than argon, its presence becomes more prevalent as the liquid argon tank for the ICP-MS depletes. Therefore, another correction can be made if we measure krypton at mass 83. Putting all the interference equations together results in an expression as follows:

$$\text{Mass 75 signal} - (3.1 \times \text{mass 77 signal} - (0.82 \times \text{mass 82 signal} - (1.0 \times \text{mass 83 signal})))$$

The signal coefficients are generated from the ratio of the relative abundances of the elements. Use of interference equations are a commonplace in routine ICP-MS analysis, however to date we know of no speciation assays that utilize them. Analysis of extracted blanks and extracted SRM's under these conditions would provide a measure of success or failure to this approach. Regardless, it is fun to think about.

Without the appropriate arsenic reference materials, identification of other arsenic compounds by the current speciation method is not possible. One option is to contact Professor K.V. Francesconi and see if he would be willing to share the four arsenosugars that are in his possession (Madsen et al., 2000). Another option would be to locate synthesis methods for several of the most probable arsenic sugars present as reported by the Kohlmeyer et al. (2002) and prepare them in-house. A third option could be to contact a chemical manufacturer and request to have the most probable arsenic sugar compounds custom made, however chances are that this option would be costly. A fourth option, would be to set-up an arsenic speciation method identical to the McSheehy and Szpunar (2000) methods and identify unknown arsenic compounds relative to the known arsenic compounds.

Addendum – Further experiments to improve speciation

The data from the above experiment indicated that the algae sample contained 10 µg/g total arsenic. This value agreed well with data obtained from independent analyses conducted for The Nature Conservancy for samples collected in Hawaii. A preliminary arsenic extraction experiment performed with trifluoroacetic acid (TFA) as the solvent and duckweed, a marine type plant in Illinois, as the sample showed some promise. However, extraction by this method on the Hawaiian algae sample was only able to recover 51% of the total arsenic. A second extraction with methanol-water performed on the raffinate from the first extraction was able to remove about 55% of the remaining total arsenic. Arsenic speciation of the extracts from these experiments by liquid chromatography inductively coupled plasma mass spectrometry (LC-ICPMS) indicated that the forms of arsenic present were not amenable to the current instrumental methods employed at ISTC. Furthermore, the extraction method was shown to cause changes in the forms of arsenic present.

Objective: To extract arsenic compounds from algae samples by a solid-liquid extraction method. To determine the arsenic species present in the algae extract and their representative concentrations by LC-ICPMS.

Sample Preparation and Total Arsenic Digestion: The sample preparation used in this experiment was similar to that detailed above. The processed sample was stored at -20°C when not in use. Total arsenic result used for calculating recoveries was based on the earlier experiment.

Extraction Methods: To prepare the algae sample for arsenic speciation analysis by LC-ICPMS, solid-liquid extractions were performed. A quarter gram of homogenized sample was treated with 5.0 ml methanol-water (3:1) at 55°C for 1 hour. The extraction solvent was collected and the procedure was repeated two more times and the extraction fluids were pooled. Eight milliliters from the pooled extract were removed and the methanol was evaporated under a gentle stream of nitrogen at 50°C. Following methanol removal, the sample was diluted to 8.0 ml with 0.2% hydrochloric acid. The final sample was then filtered through a 0.2 µ syringe filter to remove any solids. A reagent blank, an inorganic arsenic matrix spike, a certified dogfish muscle sample, and a commercially available kelp sample purchased locally were processed in parallel to verify sample extraction.

Total Arsenic Analysis: Total arsenic analysis was performed with a VG Elemental PQ Excel ICP-MS. Yttrium was utilized as an internal standard and the instrument was calibrated daily with reference materials procured from SPEX Certiprep. Verification of instrument calibration was achieved with preparations and analysis of two independent reference materials from the same vendor, but with different lot numbers. These check standards were analyzed post calibration and post sample analysis. Each ICP-MS measurement was conducted in triplicate and a sample duplicate and an analytical sample spike was performed during each assay.

Arsenic Speciation Analysis: Arsenic speciation was achieved with a liquid chromatography system interfaced to the ICP-MS instrument operated in a transient acquisition mode. The LC operating parameters were obtained from a reference method designed for marine biota (1). Separation of the arsenic compounds was achieved with a Thermo AS7 column (4mm x 250mm) with a nitric acid gradient mobile phase containing 0.05 mM benzene-1,2-disulfonic acid dipotassium salt (ion-pairing agent) and 0.5% methanol. The mobile phase flow rate was set at 1.0 ml/min and an injection volume of 30 µl was used. Yttrium prepared at a concentration of 100 ng/ml in mobile phase A was employed as an internal standard. Injection of 10 µl of the internal standard was performed post-column and is necessary since mobile phase and sample salts dampen the signal intensity over the course of the assay. Calibration of the instrument was conducted with reference materials obtained from SPEX Certiprep, Sigma, and Chem Service. Check standards were analyzed post calibration and post sample analysis.

Total Arsenic in Algae Extract Results: Total arsenic analysis of the methanol-water (3:1) extracts was achieved by ICP-MS. The average total arsenic result obtained for duplicate extract of the Hawaiian algae is 11 µg/g. The average total arsenic result obtained for duplicate extract of the kelp is 65 µg/g.

Arsenic Speciation Results: Figure 1 presents the LC-ICPMS chromatogram of the algae sample spiked with five arsenic species at a five-fold dilution. Recoveries for the five arsenic species spiked in an algae extract recovered from 84% to 106%. In addition, two unknown arsenic species were observed at retention times 6.7 minutes and 7.1 minutes. These unknown peaks were not observed in reagent blanks or calibration standards.

Figure 1
LC-ICPMS Chromatogram of Algae Extract Spiked with 5 Arsenic Species
(5-Fold Dilution)

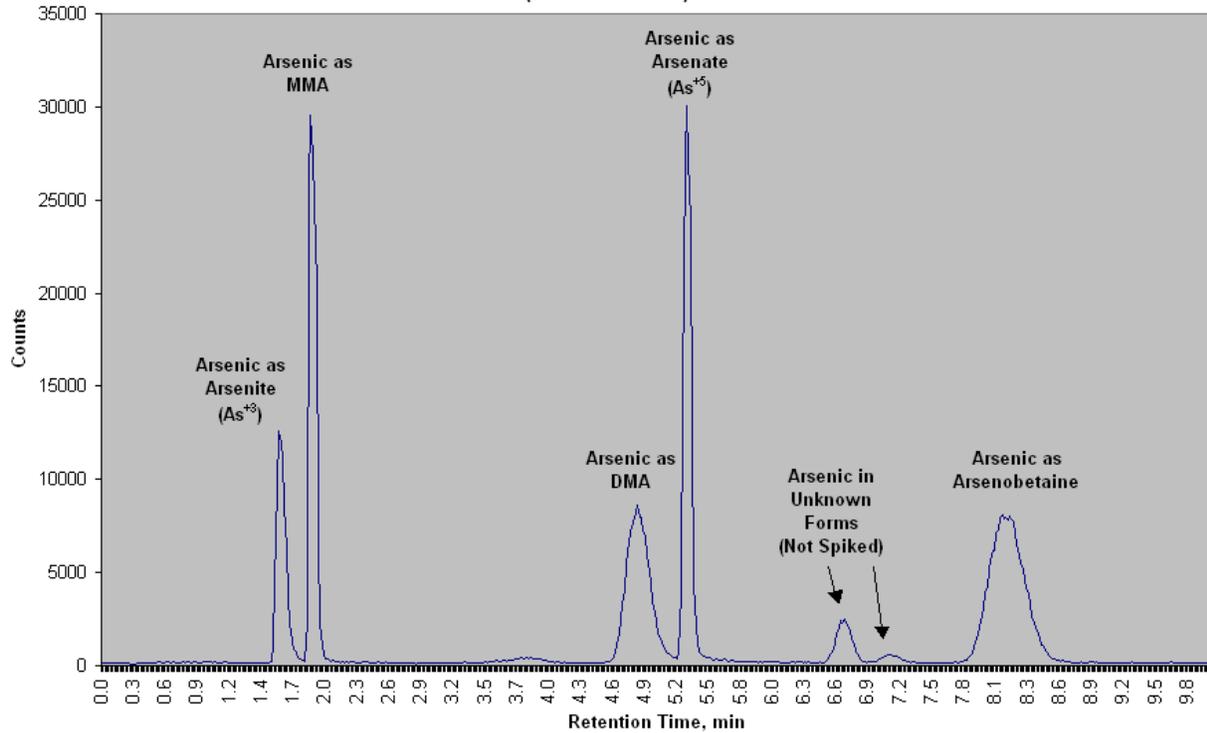


Figure 2
LC-ICPMS Chromatogram of Algae Extract
(5-Fold Dilution)

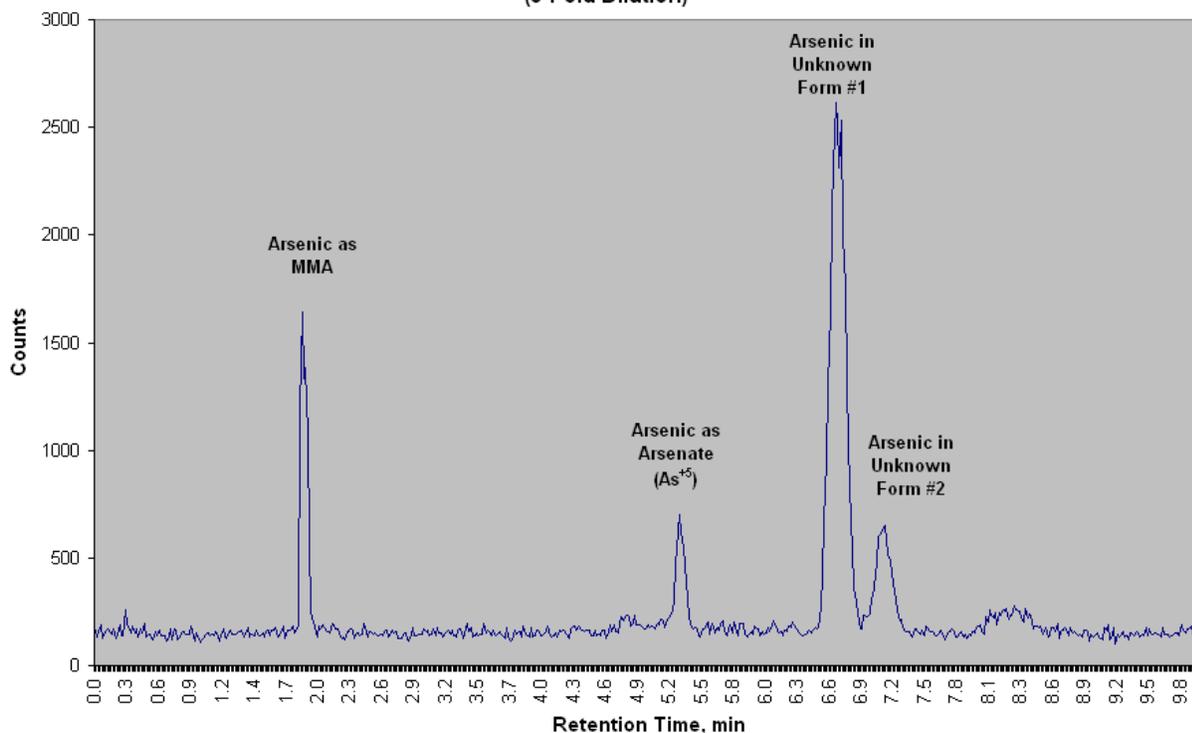


Figure 2 presents the LC-ICPMS chromatogram of an alga extract with no species spike.

The only known arsenic species observed in the extract were arsenic as MMA and arsenic as arsenate. In addition, two unknown forms of arsenic were observed in the extracts. Unknown #2 was found at the greatest concentration. Table 1 presents the final results for arsenic speciation of the algae extracts. Final results are reported as arsenic in concentration unit $\mu\text{g/g}$.

	Total Arsenic*,	Arsenite,	MMA,	Unknown Species #1,	DMA,	Arsenate,	Unknown Species #2,	Unknown Species #3,	Arsenobetaine,	Species
	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$	Sum, $\mu\text{g/g}$
<i>Gracilaria salicornia</i> (from Hawaii)	11	< 0.3	1.2	< 0.3	< 0.3	0.33	5.5	0.75	< 0.3	7.7
Kombu - Family Laminariaceae (purchased locally)	65	< 0.3	27	20	9.9	6.4	6.5	< 0.3	< 0.3	69

* - Total arsenic in extract measured by ICP-MS

Discussion: Total arsenic analysis of the methanol-water (3:1) algae extract indicates that all of the arsenic present in the algae sample was extracted. The sum of the arsenic species accounted for 70% of the arsenic present in the extract. Two known forms of arsenic were present in the algae extract. Two unknown forms of arsenic were detected in the extract. One of the unknown forms, #2, is the most abundant form of arsenic in this sample. The remaining 30% of the arsenic present in the extract is not detectable by this LC-ICPMS method.

Analysis of total arsenic in the edible kelp sample produced a total arsenic result of $65 \mu\text{g/g}$. This result is almost six times greater than the Hawaiian algae sample. The sum of the arsenic species indicates that all the arsenic present in the edible kelp was accounted for by the LC-ICPMS method. Two unknown forms of arsenic were detected in the edible kelp sample. One of the forms, Unknown #2, was identical to one observed in the Hawaiian algae sample. The concentration of this form in the edible kelp was similar to the concentration of this unknown form in the Hawaiian algae sample.

Identification of the unknown arsenic species present is impossible by these methods. In order to isolate and identify these unknown forms, separate methods would have to be developed. Edmonds, et al. were able to isolate and identify unknown forms of arsenic in the edible seaweed *Hizikia fusiforme*, however the method used were extremely labor intensive (2).

Publications Cited

- Diaz, Oscar, Yasna Tapia, Ociel Muñoz, Rosa Montoro, Dinoraz Velez, Concepción Almela (2011) "Total and inorganic arsenic concentrations in different species of economically important algae harvested from coastal zones of Chile" *Food Chem Toxicol.* 2011 Nov 25. [Epub ahead of print]
- Edmonds, E., Morita, M., Shibata, Y. "Isolation and Identification of Arsenic containing Ribofuranosides and Inorganic Arsenic from Japanese Edible Seaweed *Hizikia fusiforme*". *J. Chem. Soc. Perkin Transactions I.* 1987: 577-580
- Granchinho, S. C. R., Polishchuk, E., Cullen, W. R. and Reimer, K. J. (2001), "Biomethylation and bioaccumulation of arsenic(V) by marine alga *Fucus gardneri*". *Applied Organometallic Chemistry*, 15: 553–560.
- Kohlmeyer, U., Kuballa, J., Jantzen, E. "Simultaneous separation of 17 inorganic and organic arsenic compounds in marine biota by means of high performance liquid chromatography/inductively coupled plasma mass spectrometry". *Rapid Commun. Mass Spectrom.* 2002; 16: 965-974
- Madsen, A.D., Goessler, W., Pedersen, S.N., Francesconi, K.A. Characterization of an algal extract by HPLC-ICP-MS and LC-electrospray MS for use in arsenosugar speciation studies" *J. Anal. Atom. Spectrom.* 2000; 15: 657
- McSheehy, M., Szpunar, J. "Speciation of arsenic in edible algae by bi-dimensional size-exclusion anion exchange HPLC with dual ICP-MS and electrospray MS/MS detection" *J. Anal. Atom. Spectrom.* 2000; 15: 79
- Smith, E., Juhasz, A.L., Naidu, R. "Arsenic uptake and speciation in rice plants grown under greenhouse conditions with arsenic contaminated irrigation water". *Sci. Tot. Environ.* 2008: 277-283

USGS Award no. G12AP20133 Determination of groundwater fluxes and evaluation of water-level data to characterize effectiveness of low-permeability valley-fill deposits in the Pearl Harbor Aquifer area

Basic Information

Title:	USGS Award no. G12AP20133 Determination of groundwater fluxes and evaluation of water-level data to characterize effectiveness of low-permeability valley-fill deposits in the Pearl Harbor Aquifer area
Project Number:	2012HI412S
Start Date:	9/1/2012
End Date:	10/31/2013
Funding Source:	Supplemental
Congressional District:	Hawaii 1st
Research Category:	Ground-water Flow and Transport
Focus Category:	Groundwater, Water Supply, Models
Descriptors:	
Principal Investigators:	Aly I El-Kadi

Publications

There are no publications.

Introduction

The Pearl Harbor Aquifer is the most important aquifer on the island of Oahu and currently supplies about 100 mgd of fresh groundwater mainly for public use (Rotzoll et al., 2010). Decisions related to future infrastructure development and alternate sources of freshwater, including desalinization, will depend on the long-term sustainability of the groundwater resources in the Pearl Harbor Aquifer.

For proper resource management it is critically important to have an accurate understanding of the groundwater flow through the Pearl Harbor Aquifer. That is: (1) quantification of groundwater fluxes to the Pearl Harbor Aquifer from adjacent groundwater areas, and (2) evaluation of the effects of local hydrogeologic features, in particular low-permeability valley-fill barriers.

Stream valleys filled with alluvium below the water table act as hydrologic barriers to cross-valley groundwater flow because the deposits have a lower permeability than the adjacent basalt. Weathered basalt underneath the streambed contributes to the permeability contrast under the valley fill with respect to the otherwise high-permeability basalt aquifer. Water levels that differ by several feet on opposite sides of a valley-fill indicate an effective barrier. The effectiveness of a valley fill to impede horizontal groundwater flow depends on the geometry and hydrologic parameters of the deposits (Oki, 2005; Rotzoll and El-Kadi, 2007).

Problem and Research Objectives

The scope of work includes (1) developing a regional numerical groundwater model that quantifies groundwater fluxes to the Pearl Harbor Aquifer from adjacent areas, and (2) analyzing groundwater-level data to evaluate the hydrologic effectiveness of valley-fill barriers, including those associated with Waimano, Waimalu, and Kaluaao Streams.

Methodology

Groundwater fluxes to the Pearl Harbor freshwater-lens aquifer include surficial recharge and underflow from adjacent high-level water bodies (Schofield Plateau and dike-impounded water from the rift zones of the Koolau and Waianae Volcano). A three-dimensional island-wide MODFLOW model (Harbaugh et al., 2000) of Oahu with the focus on groundwater areas adjacent to the Pearl Harbor Aquifer will be developed. The numerical model is capable of simulating groundwater flow and the freshwater-saltwater interface using the Saltwater-Intrusion (SWI) package (Bakker and Schaars, 2005).

The model developed as part of this work will incorporate the latest available groundwater-recharge estimates developed by the USGS, recent groundwater withdrawal rates, and aquifer parameters that are based on previously published values. The steady-state model of the recent hydrologic conditions is calibrated using observed groundwater levels, vertical salinity profiles, and estimated base flows of streams. Upon successful calibration, groundwater fluxes into the Pearl Harbor Aquifer can be determined for recent conditions. The effects of predevelopment conditions on the location of the groundwater divide between leeward and Pearl Harbor side in the Koolau high-level water area can be tested.

Recent synoptic water-level surveys in the Pearl Harbor Aquifer by the USGS and water levels measured on opposite sides of valley-fills will be used to characterize the effectiveness of the alluvium as a hydrologic barrier. Moreover, continuously measured water levels will be analyzed to evaluate the cross-boundary effects of groundwater withdrawals. After removing environmental stresses that influence water levels other than groundwater withdrawals (e.g., barometric pressure, recharge events), the water-level time series can be investigated for signs of drawdown and recovery across valley fills.

Principal Findings and Significance

Study is ongoing.

Publications Cited in Synopsis

- Bakker, M., and F. Schaars, 2005, The Sea Water Intrusion (SWI) package manual part I. Theory, user manual, and examples version 1.2., University of Georgia, 37 p.
- Harbaugh, A.W., E.R. Banta, M.C. Hill, and M.G. McDonald, 2000, MODFLOW-2000, the U.S. Geological Survey modular ground-water model—User guide to modularization concepts and the ground-water flow process, U.S. Geological Survey Open-File Report 2000–92, 121 p.
- Oki, D.S., 2005, Numerical simulation of the effects of low-permeability valley-fill barriers and the redistribution of ground-water withdrawals in the Pearl Harbor area, Oahu, Hawaii, U.S. Geological Survey Scientific Investigations Report 2005–5253, 111 p.
- Rotzoll, K., and A.I. El-Kadi, 2007, Numerical ground-water flow simulation for Red Hill fuel storage facilities, NAVFAC Pacific, Oahu, Hawaii: University of Hawaii, Water Resources Research Center, prepared for TEC Inc., 74 p.
- Rotzoll, K., D.S. Oki, and A.I. El-Kadi, 2010, “Changes of freshwater-lens thickness in basaltic islands aquifers overlain by thick coastal sediments”, *Hydrogeology Journal*, 18(6), 1425–1436.

Information Transfer Program Introduction

WRRC's Technology Transfer program continued with a seminar series, project bulletins, newsletters, participation in conferences, assistance to consultants, students of all levels, and the public, participation in school science fairs, direct participation in research projects having an informational component, and an expansion and redesign of the Center's web site.

WRRC's direct audience includes the State Health Department, the State Department of Land and Natural Resources, the county water supply boards, as well as national regulatory and planning agencies. Furthermore as decision makers are strongly influenced by popular opinion, we try to educate the general public about water issues. A good deal of misinformation circulates about water resources, much of it generated by persons or groups advancing self-interested agendas. In order for our research to fulfill its potential to assist in water management it is important for the results to reach the people who can use it. That is the goal of the technology transfer effort at our Center.

Technology Transfer

Basic Information

Title:	Technology Transfer
Project Number:	2011HI317B
Start Date:	3/1/2011
End Date:	2/28/2013
Funding Source:	104B
Congressional District:	1
Research Category:	Not Applicable
Focus Category:	Education, None, None
Descriptors:	technology transfer, outreach
Principal Investigators:	Philip Moravcik

Publications

1. Moravcik, Philip, 2011, WRRRC Bulletin, 8/26/11 2 pages
2. Moravcik, Philip, 2011, WRRRC Bulletin,9/16/11 3 pages
3. Moravcik, Philip, 2011, WRRRC Bulletin,10/21/2011 4 pages
4. Moravcik, Philip, 2011, WRRRC Bulletin,1/20/12 4 pages
5. Moravcik, Philip, 2013, Water Resources Research Center Researcher Profile/Interest Guide, 2/1/13, 22 pages.
6. Moravcik, Philip, 2012, WRRRC Bulletin, 4/15/12, 4 pages.

Technology Transfer

Introduction

This is a report on the activities pursued by WRRC's Technology Transfer Office using funding from the 2011 program carried forward.

To further the goal of broadening knowledge and appreciation of Hawaii's water resources, WRRC's Technology Transfer Program produces newsletters, organizes biweekly seminars, workshops, and conferences, produces posters and other materials for presentations, and maintains the Center's website. The Program P.I. is active in meeting with agency personnel, assisting with proposal writing, research project implementation, and contributing to report authorship for the Center's research projects.

Problem and Research Objectives

This is a technology transfer project and not a research project so the "problem" and "objectives" are somewhat different than those for a research project.

The mandate of the Water Resources Research Center includes an obligation to broadly disseminate the results of its research activities to audiences of local water and wastewater agencies, environmental engineering consultants, other academic researchers, and interested members of the public.

The "problems" that this project seeks to mitigate are several; there is a lack of scientific and policy knowledge concerning water issues among Hawaii's general populace; there is a considerable amount of misinformation about water circulating in the public domain; there is a lack of understanding and appreciation of the value of water research conducted at the University among policy makers and governmental agencies in the state. We seek to redress these problems through our outreach/educational activities. Our objective is to inform the public and governmental agencies to improve the understanding and management of water resources in Hawaii and the region.

Methodology

The Technology Transfer Office employs a range of media to disseminate the results of research done at the Center. WRRC bulletins; other publications; web site; workshops, meetings, and conferences; and regular biweekly seminars all served to aid the center in transferring information concerning water-resource research and issues to its audience.

WRRC's Technology Transfer program included a seminar series, project bulletins, newsletters, participation in conferences, assistance to consultants, students of all levels, and the public, participation in school science fairs, direct participation in research projects having an informational component, and an expansion and redesign of the Center's web site.

Technology Transfer Program Activities

Under this no cost extension to the 2011 WRRIP Technology Transfer grant made to the University of Hawaii's WRRC, the Center's Technology Transfer Specialist attended a meeting at the Kauai Community College (KCC). During this meeting discussions were held with the College's administration and interested faculty members with the purpose of identifying possibilities for collaboration and participation in joint activities. KCC is engaged in a number of very interesting sustainability projects that the faculty and students have been conducting with minimal financial resources. Ho'ouluwehi: The Sustainable Living Institute of Kauai was founded at Kauai Community College in 2008 by an idea fostered as seeds of inspiration from the platform of Chancellor Dr. Helen Cox with the help of her faculty and staff. WRRC's Technology Transfer Specialist and other members

of the WRRC faculty met with Chancellor Cox, and an informal agreement to further pursue avenues of collaboration between KCC and WRRC was made.

Other activities supported by the no cost extension included poster production, organization of a meeting of the Center's Advisory Council, printing of the Center Faculty Profile book, and repair and replacement of computer hardware.

Technology Transfer

Basic Information

Title:	Technology Transfer
Project Number:	2012HI375B
Start Date:	3/1/2012
End Date:	2/28/2014
Funding Source:	104B
Congressional District:	1
Research Category:	Not Applicable
Focus Category:	Education, None, None
Descriptors:	technology tranfer, outreach
Principal Investigators:	Philip Moravcik

Publication

1. Moravcik, Philip 2012, Water Resources Research Center - Researcher Profile - Interest Guide, internal publication of the University of Hawaii Water Resources Research Center, 1-20

Technology Transfer

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Technology Transfer Program Activities

- **Organization of Seminars**

As it has done for more than twenty years the Technology Transfer Program continues to organize biweekly seminars designed to foster communication among WRRC researchers, students, and the organizational target audience of government agencies, private-sector researchers, and members of the general public with an interest in water-resource issues. Each semester one WRRC faculty member is appointed to organize the seminars with the assistance of the Technology Transfer office, and recruit speakers from university faculty, visiting scientists, government agencies, and private sector firms. Topics thus vary depending on the interests of the coordinator and availability of speakers. Typically the seminars include reports on WRRC projects and discussions by government officials on emerging water-related issues. The seminars are generally well attended and provide one of the few public forums

in the state for the discussion of water issues. The following is a list of the twenty-two seminars presented during the reporting period.

Spring Semester 2012

Mar. 3, 2012	<i>Microbial Protection against Plant Disease: From Biochar to Biocontrol Agents</i>	Eddie Cytryn, Institute for Soil, Water, and Environmental Sciences, ARO, Volcani Agriculture Research Center, Bet Dagan, Israel
Mar. 7, 2012	<i>Digging a Little Deeper – Designing Green Roofs</i>	Dawn Easterday, ASLA, GRP, LEED AP Senior Landscape Architect, Belt Collins Hawaii LLC
Mar. 14, 2012	<i>Can Interdisciplinary Centers work? The University of Minnesota Institute on the Environment as Object Lesson</i>	Deb Swackhamer, Professor and Co-Director, Minnesota Water Resources Center; Environmental Health Sciences, University of Minnesota
Mar. 21, 2012	<i>The Landscape Inventory, Cross-Disciplinary Uses of a Mapping Project for Water Resource Management on Campus</i>	Austin Stankus, University of Hawaii, Department of Zoology
April 4, 2012	<i>Turfgrass Management</i>	Jordan K. Abe, Superintendent – Ala Wai Golf Course, Dept. of Enterprise Services
April 18, 2012	<i>Exploring Subsurface Fluid Flow and Solute Transport by HYDRUS 1, 2/3D</i>	Seo Jin Ki, Researcher, Water Resources Research Center, University of Hawaii
May 2, 2012	<i>Halorespiration a Natural Process</i>	Paige Novak, University of Minnesota, BioTechnology Institute
June 13, 2012	<i>Fast Track/ Rapid/ Automated Methods to detect Microorganisms</i>	Dan Fung, Professor of Food Science Dept. of Animal Sciences and Industry, Kansas State University, Manhattan, Kansas

Fall Semester 2012

Sept. 5, 2012	<i>Update on the Mission, Progress and Issues Facing Honolulu Board of Water Supply</i>	Ernest Lau, Manager and Chief Engineer, Honolulu Board of Water Supply
Sept. 19, 2012	<i>An Overview of the City's Storm Water Program</i>	Gerald Takayesu, Branch Head, Storm Water Quality Branch, Dept. of Environmental Services, City and County of Honolulu
Oct. 3, 2012	<i>Water Issues in Hawaii: A Survey on Public Attitudes</i>	Luisa Castro, Education Specialist-Assistant Water Qual. Coordinator Maui Agricultural Research Center
Oct. 10, 2012	<i>Sustainability Analysis and Case Study of Transportation Modes and Island Communities</i>	Panos Prevedouros, UH Manoa, Civil and Environmental Engineering
Oct. 17, 2012	<i>Watershed Management in Hawaii Volcanoes National Park: An Environment Without</i>	Dr. Rhonda Loh, Chief of Natural Resources Management,

	<i>Surface Water</i>	Hawaii Volcanoes National Park
Nov. 7, 2012	<i>Report on 2012 International Water Association (IWA) Sponsored, "Ecotechnologies for Wastewater Treatment Conference"</i>	Dr. Roger S. Fujioka, PhD., Emeritus Researcher, Water Resources Research Center, University of Hawaii
Nov. 21, 2012	<i>UV Disinfection Guidelines (3rd ed.)</i>	Dr. Victor Moreland, Water Resources Research Center, University of Hawaii at Manoa

Spring Semester 2013

Jan. 23, 2013	<i>Revision of a Regional Pesticide Leaching Tool in Hawaii for Volatile Organic Compounds</i>	Seo Jin Ki, Researcher, Water Resources Research Center, University of Hawaii
Feb. 5, 2013	<i>An Integrated Media, Integrated Processes Hydrological Model and its Application to Watershed Water Quantity and Quality Simulation</i>	Fan Zhang, Professor, Institute of Tibetan Plateau Research Chinese Academy of Sciences
Feb. 6, 2013	<i>Revision of a Regional Pesticide Leaching Tool in Hawaii for Volatile Organic Compounds</i>	Dr. Joseph D. Rouse, Associate Professor of Water Resources Environmental Engineering, WERI, University of Guam
Feb. 7, 2013	<i>Finding the Needle in the Water Column</i>	Stephaney D. Leskinen, Research Associate, Univ. of South Florida
Feb. 11, 2013	<i>Measurement of Health Risks Based on Fingerprinting Microbial Populations in Sewage, Coastal Waters, and Streams in Hawaii</i>	Marek Kirs, PhD, Assistant Researcher, University of Hawaii, Water Resources Research Center
Feb. 12, 2013	<i>Pathogens in Water: Value & Limits of Correlation with Microbial Indicators</i>	Pierre Payment, Professeur Institut Armand-Frappier, Laval, Québec, Canada
Feb. 20, 2013	<i>Roadmap for Best Practices in Microbial Risk Assessment of Water</i>	Yong Jin Lee, Yong Jin Lee, Postdoctoral Research Associate Institute for Environmental Genomics University of Oklahoma

- **WRRC Website**

The Center's website (www.wrrc.hawaii.edu) is continually updated with new information about WRRC researchers' activities, seminars, reports, meetings, grant announcements, scholarship opportunities, etc. The site provides information about center facilities and personnel as well as a database of WRRC publications. A search function provides easy access to the available information. There is a link on the Center's home page that leads to an archive of full-text PDF files of reports written by WRRC researchers since the early days of the Center. This permits extremely easy access to our reports for our clientele. Following a decision by our past director WRRC no longer publishes reports in-house and our researchers submit their reports as articles directly to journals which generally restrict access to these articles. WRRC continues to post the abstracts and publication information about these articles on our website.

- **Poster Production**

The Technology Transfer Program PI assisted numerous center faculty and graduate research assistants in the design and production of posters illustrating research projects for display at meetings and conferences. Several graduate-research-assistant posters were recognized by conference awards during the reporting period. Media Contact During the reporting period the Technology Transfer project P.I. responded on several occasions to inquiries from reporters about water and environmental issues. In addition the Technology Transfer Office submitted news releases regarding the research activities of Center faculty to local and national media through the University of Hawaii's media office.

- **L. Stephen Lau Scholarship**

The Technology Transfer Office took responsibility again this year for coordinating the announcement, application review, applicant selection for the L. Stephen Lau Scholarship. This scholarship is made annually thanks to an endowment by former WRRC Director L. Stephen Lau and his wife.

- **Committee to select WRRIP 104b grantees**

The Technology Transfer Specialist at WRRC served on the committee to review and select for funding proposals made under the WRRIP 104b program.

- **Organization of Center's Advisory Council meeting**

The Technology Transfer Office organized a meeting of the Center's Advisory Council. This meeting included presentations by all WRRC core faculty and by current WRRIP 104b grantees.

Members of the Center's Advisory Council (or their representative) in attendance included:

Hawaii Water Resources Research Center Advisory Council 2012	
Name/Title	Agency
Ernest Lau, Manager and Chief Engineer	Honolulu Board of Water Supply
Ross Tanimoto, Deputy Director	Department of Environmental Services, City and County of Honolulu
Debbie Solis, Program/Project Manager - Civil Works staff	U. S. Army Corps of Engineering
Roy Hardy	State of Hawaii, Commission on Water Resource Management
Thomas Matsuda, Program Manager, Pesticide Branch	Hawaii Department of Agriculture
J. Mark Ingoglia, Chief, Environmental Branch	HQ PACAF, US Airforce
Scott McAdam, President, HWEA Hawaii Section	Hawaii Water Environment Association
Richard Cox	Private Citizen, former Water Commissioner
Stephen Anthony, Director,	USGS Pacific Islands Water Science Center
Dayan Vithanage	Oceanit Inc. consultants in Honolulu

For American Samoa	
Daniel Aga, Dean and Director of Community and Natural Resources Division	American Samoa Community College
Robert Kerns, Senior Environmental Engineer	American Samoa Power Authority
Jason Gambatese, Program Manager	USEPA Region 9
Faamao Asalele, Jr., Asst. Director	American Samoa EPA

- **Establishment of the American Samoa Water Center**

The Technology Transfer Specialist at WRRC played a major role in expediting the establishment of the new WRRIP Center for American Samoa. The activities included expediting communication between the Governors of Hawaii and American Samoa, and contacting and securing the participation of American Samoa Community college and other entities dealing with water in American Samoa.

- **WRRC affiliates publication**

The Technology Transfer Office was instrumental in the identification of a cadre of affiliate academic researchers to collaborate with WRRC. We identified, contacted, and secured agreements from some 75 faculty members at the University of Hawaii and other Hawaiian educational institutions to collaborate on future research projects. The hope is to leverage the large range of expertise available in academia in Hawaii in obtaining research grants and addressing various environmental issues.

The Technology Transfer Office produced a publication that lists these affiliates, their expertise, interests and contact information to distribute to prospective funders and thus inform them as to the expertise the Center is able to bring to bear in addressing research needs.

- **Media Contact**

During the reporting period the Technology Transfer project P.I. responded on several occasions to inquiries from reporters about water and environmental issues. In addition the Technology Transfer Office submitted news releases regarding the research activities of Center faculty to local and national media through the University of Hawaii's media office.

- **RWQC conference**

The Technology Transfer Office assisted with the organization of the U.S. Recreational Water Quality Criteria: A Vision for the Future held in Honolulu, March 11-13, 2013. Among other work, the Office produced a website for the conference. <http://www.wrrc.hawaii.edu/rwqc2013/>

- **Oversight of Center publications staff**

The Technology Transfer Office provides oversight of the Center's publications staff, which assist faculty with layout and production of articles and reports.

- **Editing**

WRRC's Technology Transfer Specialist provided editorial services for numerous reports and articles during the reporting period. This work helps to disseminate the Center's research results through journals and other publications.

USGS Summer Intern Program

None.

Student Support					
Category	Section 104 Base Grant	Section 104 NCGP Award	NIWR-USGS Internship	Supplemental Awards	Total
Undergraduate	1	0	0	0	1
Masters	2	0	0	0	2
Ph.D.	4	0	0	0	4
Post-Doc.	3	0	0	2	5
Total	10	0	0	2	12

Notable Awards and Achievements

Olivia Schubert, 2011, Best Student Poster for “Long-term aspects of mountain rainfall and vegetation change, O’ahu,” at 2011 Tester Symposium at the University of Hawaii at Manoa

Reshaping the Regulatory Framework for Hawaii Aquaculture – Water Quality Standards, Coastal Fishponds, and Shellfish Ground’s student trainee, legal research assistant Mele Coleman, was awarded a National Sea Grant College Program Dean John A. Knauss Marine Policy Fellowship. She left the project in January 2013 to begin serving a one-year fellowship term with NOAA in Washington, D.C. Upon her return to Hawaii, Ms. Coleman will serve as a law clerk for Judge Daniel R. Foley at the State of Hawaii Intermediate Court of Appeals.

Publications from Prior Years