

**Water Resources Research Center  
Annual Technical Report  
FY 2013**

# Introduction

When compared to population centers in continental areas, water shortages and quality problems are more critical in Hawaii and the Pacific because of their geographic isolation and small land areas. Owing to their spatial and temporal scales, the Hawaiian Islands can serve as a microcosm of what may already be a reality for many areas in the world that share similar water supply and quality problems. Over the past 50 years, since WRRC was established, the research focus mainly addresses the following general categories.

- Groundwater Characterization Assessment and Modeling
- Groundwater Contamination, Drinking Water Supply Protection
- Recreational/Microbial Water Quality, Microbial Methods
- Wastewater Treatment Technology
- Wastewater Reuse/Disposal
- Watershed/Non-Point/Runoff
- Ocean Outfall Biomonitoring
- General Marine Water Quality
- Economics/Policy/Law
- Climate/Atmosphere/Precipitation/Evaporation/ Flooding
- Rainwater Catchment
- Streams/Lakes

In addition to basic research, many of the studies have aimed at solving specific water resource problems, such as biomonitoring near ocean outfalls, arsenic contamination, and rise in well nitrate level. Others have been initiated to meet federal regulations/obligations, including biomonitoring, source-water assessment program, and watershed protection plan.

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The Hawaii NIWR 104-B program for FY 2013 funded 8 new research projects 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. This report covers studies completed or in progress during the reporting period. Subjects cover, in general, water resources sustainability, climate and climate change influence on water resources, chemical and microbial water quality, modeling, and parameter estimation.

The Hawaiian Islands have one of the most diverse rainfall patterns on earth. The mountainous terrain, persistent trade winds, heating and cooling of the land and the regular presence of a stable atmospheric layer at an elevation of around 7,000 ft interact to produce areas of uplift in distinct spatial patterns anchored to the topography. The resulting clouds and rainfall produced by this uplift lead to dramatic differences in mean rainfall over short distances. Knowledge of the rainfall patterns is critically important for a variety of resource management issues, including groundwater and surface-water development and protection, controlling and eradicating invasive species, protecting and restoring native ecosystems, and planning for the effects of global warming. A project was aimed at updating the set of month-year rainfall maps, to extend the available rainfall record through 2010 to enable analyses of more recent hydrological conditions in Hawaii. The maps were developed for the islands of Kauai, Oahu, Molokai, Lanai, Maui, and Hawaii, 2008–2010. All rainfall station data from over 10 networks has been collected, formatted, and quality controlled (including standardizing units so all networks could be combined into one file). Data for 2007 were also collected so the new station data can be matched with previous records. Many networks use unique identifier numbers for their stations, so work has been done to match these stations with standardized State Key Numbers (SKNs). Most of the data from these networks were available online for downloading and processing, however, some data from the State Climatologist office had to be hand entered. These data were entered twice so that the two datasets could be compared and errors removed. One of the islands' recent data was not available in the State Climatologist office, and therefore had to be located and hand entered. For new stations (not previously recognized by the State Climatologist), new SKNs have been assigned. A master list of the currently operating stations, along with their metadata, has been compiled.

A project covered during the reporting period dealt with forecasting climate change impacts on watershed-based ecosystem services in Hawaii. Climate change is expected to have significant impacts on native, threatened and endangered bird species, particularly in terms of habitat alteration. Understanding and modeling these impacts in a manner that is useful for decision-makers or management, however, remains difficult since many empirical modeling frameworks require large amounts of long-term data that can be costly to collect. To address this, the study proposes a decision support approach towards understanding climate change impacts on the habitat, life history functions, and abundance of endangered bird species by coupling regional watershed simulation models (e.g., AnnAGNPS) under Intergovernmental Panel on Climate Change defined scenarios and expert knowledge-based models of bird ecology. To test the approach, the study uses data from the Hawaiian Stilt, Hawaiian Coot and Hawaiian Moorhen populations, and watershed data from the island of Kauai as a case study. The type of decision framework proposed provides wildlife managers a low cost approximate understanding of the dynamic interaction of climate change, habitat and wildlife ecology based on pooling available expert knowledge and existing data about watershed projections.

Climate change was also a factor in a study that is concerned with the fact that stream flow in Hawaii and throughout the tropics is currently threatened by a number of factors. These factors include reduced precipitation due to climate change, water diversions, and invasive plant species. However, little is known of how aquatic macroinvertebrates are responding to these changes. Decreased stream flow can reduce body conditions and reproductive output of native 'opae shrimp (*Atyoida bisulcata*) in streams on Hawaii Island, but the underlying mechanisms leading to these changes have yet to be identified. Furthermore, these impacts

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may be confounded by competition between shrimp and the exotic caddisfly (*Cheumatopsyche analis*). This study will compare food webs in five streams along a rainfall gradient (3,000–7,000 mm/year) on the North Hilo coast of Hawaii Island. This unique system provides a space-for-time substitute to investigate the effects of decreased stream flow on the diets of macroinvertebrates. Stable isotope signatures from dominant food resources and consumers are being used to create a mixing model that will contrast food web structures between streams.

Another climate related project dealt with the long-term aspects of high-elevation rainfall and climate change on Oahu. The climate and precipitation regime of the North Pacific and Hawaii varies substantially across timeframes from annual to decadal and longer, and includes ENSO- and PDO-scale dynamics. Water resource planning 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 Oahu. This study uses vegetation histories and organic geochemistry, including stable isotopes from mountain peat swamp sediments at multiple sites, to better understand the intensity and frequency of dry climate periods in Hawaii. This work will bridge important gaps in hydroclimate and climate change knowledge for Hawaii and the Pacific, and serve as a platform for additional NSF funds.

An additional climate-related project deals with evaluating the impact of drought conditions upon the Waiahole Ditch System Development Tunnels on Oahu, with the aim of assessing groundwater sustainability under adverse climate change conditions. For the study area, reductions in the base-flow discharges of the high-level development tunnels of the system are believed to be associated with regional recharge distribution shortfalls to the vast and heavily utilized Pearl Harbor aquifer. Such a distribution may be observed by shallow index monitor wellhead-level responses located within distinct hydrologic sectors of the Pearl Harbor aquifer. The objective of this study is to define the relationship between high-level dike compartments and the down-gradient recharged Pearl Harbor basal aquifer. Field verification of presently measured flume and weir discharges of the Waiahole Ditch System development-tunnels are conducted to assess the efficacy of these devices in providing accurate tunnel discharge accounting. The extensive compiled data will be used for future research to study the overall sustainability of the study area. In addition, base flow discharges will be evaluated by using climatological data during past episodes of drought. Univariate multiple regression models will be used to evaluate the relationship among predictor variables (rainfall distribution, tunnel base flow discharges, Pearl Harbor groundwater production), and response variable (monitored wellhead levels). Semi-partial correlations will be used to identify the unique contribution of predictor variables upon a response variable. The relationship between high-level dike compartments and the down-gradient recharged Pearl Harbor basal lens will offer an insight into future adverse climate changes upon groundwater sustainability as framed through a retrospective analysis of significant drought episodes. This will benefit policy makers in preparation for diminishing sustainable yields among high levels sources and the Pearl Harbor aquifer. Owing to the potable quality of water from high-level Waiahole tunnels, consideration to its utility and sustainability as a future municipal resource may also represent an option to costly groundwater treatment of plantation-era fumigants found in Pearl Harbor groundwater sources.

A study also related to the Pearl Harbor Aquifer concerns determining groundwater fluxes and evaluating the effectiveness of low-permeability valley-fills in the aquifer's area. Such an aquifer is a very critical source considering that it provides the majority of groundwater used on Oahu. Successful water-resource management therefore needs an accurate understanding of the groundwater flow through the Pearl Harbor Aquifer and quantification of groundwater fluxes to the aquifer from adjacent groundwater areas. Accurate assessment also requires evaluating the effects of local hydrogeologic features, in particular low-permeability valley-fill barriers including those associated with Waimano, Waimalu, and Kaluaao Streams. A regional numerical groundwater model was developed to address these needs. Oahu's complex geologic structure was reduced to three hydrogeologic units: 1) dike-intruded volcanic rock, 2) dike-free volcanic rock, and 3) sediments and rejuvenated Volcanics. The altitude of the contact between overlying sediments and volcanic rock was generated to facilitate model grid generation. Measured water levels on Oahu from various sources

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were compiled to calibrate the model. Results are not final because the USGS recharge estimates of Oahu have only been recently made available.

Water quality was studied regarding the fate and transport of pharmaceutically active compounds in a simulated riverbank filtration (RBF) system. The occurrence of pharmaceutically active compounds (PhACs) in environmental waters has drastically increased during the past few decades. Riverbank filtration can be a possible method to remove PhACs. The objectives of this study were to investigate the impact of different levels of oxygen, variable organic matter content of the source water, seasonal variability of temperature, occurrence of air below the clogging layer on the dynamics of redox conditions and on the removal of selected PhACs. The behavior of six PhACs (caffeine, carbamazepine, 17- $\beta$  estradiol [E2], estrone [E1], gemfibrozil, and phenazone) was evaluated by column experiments and using a sandbox consisting of two side-by-side rectangular boxes. The study indicated that RBF can be effectively used to remove most of the PhACs present in surface waters. However, the geochemistry of the RBF site is expected to play a key role in their removal. Depending on the compound, removal of PhACs may predominantly occur due to biodegradation; however, environmental variables, such as oxygen and temperature may enhance or limit biodegradation. Limited and slower removal of selected PhACs may occur during the winter temperature conditions. Limited removal of caffeine (< 10%) and gemfibrozil (< 30%) occurred regardless of the different environmental conditions. Among the different PhACs, removal of phenazone occurred only under aerobic conditions, while removal of caffeine was highly impacted by the level of organics as well as by the temperature. The occurrence of air beneath the riverbed can enhance the development of locally present aerobic conditions which lead to an enhanced removal of redox sensitive PhACs.

Hawaii faces immediate challenges in meeting the demand for safe drinking water and the management of wastewater. 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. During this reporting period, a water literacy project dealt with 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, the study focused on educating the University of Hawaii at Manoa campus community to raise literacy about water-related issues at the campus, neighborhood, watershed, and island scales.

This report covers three microbiology-related projects. The first addresses sewage contamination of Nawiliwili Streams and Kalapaki Beach. Analyses of 117 water samples indicate that elevated indicator bacteria concentrations in the Nawiliwili watershed appear to originate from environmental sources. A human sewage marker was detected at several locations in the watershed, including the sites upstream from the Lihue wastewater treatment facility, indicating that leaking sewer and/or on-site septic systems may contribute to the watershed impairment. The water quality at the Kalapaki Beach was compliant with the Hawaii recreational water quality standards.

The second, ongoing project dealt with evaluating rapid qPCR method for enterococci with correlative assessment for molecular markers for sewage-contamination in selected environmental water samples from Hawaii. Sample collection is complete. Analyses of 155 coastal water samples (collected 05/14/2013 to 04/08/2014) indicates relatively good microbial water quality at the 12 beach studied, while analyses of 117 Manoa watershed samples (collected 05/21/2013 to 04/21/2014) strongly suggest human sewage-related impairment of the urbanized section of the Manoa Stream. Evaluation of microbial source tracking markers in sewage and fecal samples indicates that both human-sewage specific markers tested (Polyomavirus and Bacteroides), are well suited for contamination source partitioning in Hawaii. Decay rates of both markers were comparable in marine and freshwater microcosms, advocating usage of those markers in both environments. Molecular signal from human specific Bacteroides decays roughly twice as fast as cultivable enterococci, which in turn decays roughly twice as fast as human Polyomaviruses. Coastal samples, extracted and purified using commercial kit, contained no inhibitors, while 6.8% of the samples collected from the

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Manoa Stream contained PCR inhibitors, which if not accounted for, could lead to bias in concentration estimates ( $\geq 1$  log). All samples collected will be analyzed using USEPA Method 1611 (rapid DNA extraction combined with qPCR for enterococci) in the summer of 2014.

The final microbiological project concerns characterization of microbial communities from wastewater plants and selected beaches of Hawaii using a metagenomics approach. This ongoing project utilizes next generation sequencing technology to provide a more complete view of the coastal ecosystem than can be provided by analysis of cultivable bacteria. Sample collections from wastewater treatment plants (Hawai Kai, Honouliuli, Kailua, and Sand Island), six sites around Sand Island outfall, and nine sites in the Manoa watershed are complete and have been analyzed for enterococci and on three different culture media. Over 1000 environmental isolates have been stored for further analyses. Two hundred thirty one filters will be used to determine microbial community composition by next generation sequencing.

Another project acquired sedimentation data towards promoting reservoir sustainability and advancing watershed science. Hawaii's reservoirs face growing scrutiny due to heightened dam safety and flood control concerns, increasing water demands, and uncertain water pollution effects. In order to promote long-term reservoir sustainability, it is vital that we improve our understanding of reservoir capacity loss due to sedimentation. This project collected, organized, and analyzed existing physical data about reservoirs located on the main Hawaiian Islands. Information was obtained by interviewing reservoir managers on the importance of reservoir sedimentation that would affect the reservoir's capacity, and if they were interested in future survey work to understand the rate of sedimentation. Results showed that although the sedimentation rate is not documented, it is a serious concern and challenge for reservoir management. Five different management types were identified—federal, state, county, private agriculture, and private development. Management practices and reservoir maintenance differed significantly between these groups. Sedimentation, in some cases, was significant enough to lead to dry reservoir conditions. However, unlike in other parts of the world where reservoir sedimentation can be directly correlated with watershed erosion practices, in Hawaii, most reservoirs are partial diversions from streams or from irrigation water systems that are far separated from the watersheds above the reservoir. While engineering surveys consider the structural features of the dam, few also include information about capacity loss. The study recommends that dual-frequency bathymetric surveying equipment would provide accurate assessments of sediment depths. Reservoirs in Hawaii are a significant resource for the state's water supply, and maintenance, including assessment and removal of sediments, might be an important consideration for future discussion.

A water-quality monitoring study advanced the use of engineering palm peroxidases for wastewater treatment and water pollution monitoring. Peroxidases are a large family of enzymes that typically catalyze an oxidation reaction utilizing peroxides, such as hydrogen peroxide. Windmill palm peroxidase is one of the most stable peroxidases, which possesses ultrahigh thermal stability (up to 90 °C) and extraordinary pH tolerance (pH 2 to 11). In this project, the peroxidase gene was cloned from palm tree leaves. Eleven mutations to the native palm peroxidase gene were designed based on the glycosylation sites of the native peroxidase and then created. The genes were transformed into yeast (*Pichia pastoris*) for expression. Of the eleven mutations, two yeast mutant strains produced a peroxidase that was highly active and stable. The engineered palm peroxidase has a His-Tag at the C-terminus of the amino acid sequence, which facilitates the palm peroxidase purification. The engineered peroxidase was purified and immobilized by Ni<sup>2+</sup>-nitrilotriacetic acid (NTA) derivative functionalized carbon coated cobalt magnetic nanoparticles (Co/C NPs). Two new custom-made bioreactors were designed and manufactured. Each bioreactor has a pH probe, a DO probe, a temperature probe, and a motor for automatic stirring. Utilization of the bioreactor for palm peroxidase expression is being undertaken.

Accurate estimates of regional hydraulic properties are essential for models that are used to manage groundwater availability and quality. Aquifer hydraulic properties can be derived from tidally influenced groundwater levels that are recorded in wells and coastal pools. This is possible because the attenuation of

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tidal signals with distance inland from the coast reflects the aquifer's regional hydraulic properties. Localized studies of tidal attenuation in Hawaii have been published, but no regional synthesis of tidal attenuation information in Hawaii exists. A study covered by this report has estimated aquifer properties from tidal attenuation for Hawaii using groundwater-level records from more than 250 wells in basalt and more than 200 wells in coastal sediments. Results were combined in a regional context with respect to the hydrogeologic framework of each island in Hawaii. The analysis can improve conceptual models of groundwater flow in Hawaii aquifers and facilitate the development of regional numerical groundwater-flow and transport models needed for sustainable water-resource management. The results are implemented in an ongoing USGS regional volcanic-rock aquifer study.

A comprehensive study is addressing groundwater sustainability of the Island of Tutuila, American Samoa, where the continued sustainability of groundwater resources is in question. To date, there are no sustainable yield estimates for the island's aquifers and anthropogenic sources of nutrients and other contaminants represent a serious threat to groundwater, surface-water, coastal waters, and coral reefs. This study is currently focused on producing a better understanding of the island's hydrogeological system as protection of water resources is critical in a time of growing water demand, continued well salinization problems, and EPA mandated shutdowns of contaminated wells. The current water distribution system, which is reliant on the high yielding, but contamination prone Tafuna-Leone plain aquifer, will likely not continue to support the needs of the population. However, high-level groundwater resources may be able to provide a sustainable future supply. The study is currently developing numerical models for use in answering questions pertaining to well salinization, climate susceptibility, potential for development of new groundwater resources, and contaminant transport within groundwater aquifers. Preliminary results identify anthropogenic sources of nutrient contamination underlying heavily populated areas. Future work during a field campaign in summer 2014 will include well testing, continued water sampling, and gathering of data for better model calibration. Data analysis and model development will continue throughout 2014.

# Assessing Ground Water Sustainability of the Island of Tutuila, American Samoa

## Basic Information

<b>Title:</b>	Assessing Ground Water Sustainability of the Island of Tutuila, American Samoa
<b>Project Number:</b>	2013AS424B
<b>Start Date:</b>	3/1/2013
<b>End Date:</b>	2/28/2014
<b>Funding Source:</b>	104B
<b>Congressional District:</b>	2
<b>Research Category:</b>	Ground-water Flow and Transport
<b>Focus Category:</b>	Water Quality, Hydrogeochemistry, Non Point Pollution
<b>Descriptors:</b>	None
<b>Principal Investigators:</b>	Aly I El-Kadi, Henrieta Dulaiova, Craig R Glenn

## Publications

There are no publications.

## **Introduction**

The island of Tutuila in the territory of American Samoa is currently faced with two critical challenges that threaten the sustainability of domestic water supplies: water quantity and water quality. Groundwater sources provide nearly all drinking water to the island's ~57,000 residents, and a number of current sources are suspected to harbor contamination from pesticide residues, pollutants associated with automobiles, and pathogen and nutrient pollution from poorly constructed human and pig waste disposal systems (ASEPA 2006). The island can be divided into two main geographic regions, 1) the mountainous-forested interior, and 2) the geologically recent and thus, very hydrologically conductive Tafuna-Leone Plain (Fig. 1). In the Tafuna-Leone Plain, septic tanks, cesspools, and piggeries lie proximal to the drinking water wells in a highly permeable unconfined aquifer (Davis 1963). The negative impacts on groundwater quality are obvious and an understanding of the effects of human development on the sustainability of this resource is essential to water managers that are currently faced with hard decisions.

## **Problem and Research Objectives**

There are currently no established aquifer boundaries or estimates of sustainable groundwater yields for the island of Tutuila. Definitions of aquifer zones based on geologic information and groundwater geochemistry can be used along with regional and local scale MODFLOW and SEAWWAT models to make estimates of sustainable yields. If high-level groundwater data is available for model calibration purposes, an assessment of the developability of the resource may also be possible.

To date, no assessments of the effects of land use on Tutuila's groundwater quality have been made. By comparing the density of potentially contaminating activities in well capture zones, a preliminary determination of the potential for domestic and agricultural contamination at current or future well sites can be made.

The effects of future climate uncertainty are inescapable and will have implications on water resource management throughout the Pacific. Using the data from the climate model predictions as input parameters for groundwater models will allow us to make recommendations to water managers that account for a variable climate.

## **Methodology**

Groundwater modeling is invaluable for good management and proper planning of water resource development. New water level and climate data has been collected is currently being integrated into a MODFLOW model. Once calibrated, local and regional models will be applied to address a number of specific questions. Due to their degree of spatial and temporal dimensionality, numerical models have proven to be more effective in defining and estimating values of sustainable yield than in analytical models such as RAM2 (Liu and Dai 2012; El-Kadi 2013). Geochemical information from well sites can be used to assess the degree of anthropogenic effects on groundwater, as well as provide insight into the sources of both water

and nitrate. Stable nitrogen isotopes of groundwater nitrate are widely used to differentiate between fertilizer versus animal/human and soil nitrate (Kendall and McDonnell 1998, and papers therein). On other tropical islands, stable isotopes of water have been used to assess recharge altitude (Scholl 1996) as well as the seasonality (Davis 1970) of precipitation contributing to groundwater. Coupled with other indicators, such as radon gas concentration and turbidity readings, timescales for precipitation, to surface water, to groundwater fluxes can be constructed (Le Gal et al. 2001).

## **Principal Findings and Significance**

A field expedition to Tutuila for site reconnaissance and sampling was completed in summer 2013. Preliminary water samples from wells, springs, and streams were collected and the extent of available well data was determined. Nutrient concentrations were analyzed and the Tafuna-Leone Plain region was found to have a distinctive excess of nitrate and total nitrogen as compared to the small valley fill aquifers that are distributed around the coast (Fig. 2). Stable isotopes of N in nitrate were analyzed with the denitrifier method (Sigman 2001) and values from Tafuna-Leone Plain samples indicate groundwater nitrate inputs from septic or piggery waste sources (Fig. 3). Analysis of stable water isotopes were analyzed and the results indicated that enrichment in heavy isotope ratios is found in the eastern portion of Tutuila but not in the western portion of the island. Possible causes could be either 1) rain out as clouds move over Tutuila from east to west (the prevailing wind direction), or 2) seasonal variation in isotopic ratios coupled with a significant difference in hydrologic conductivities of the aquifers in each region. These findings are encouraging for continued sampling of precipitation and groundwater to help constrain the rate at which surface water becomes groundwater.

## **Future Work**

A sampling campaign to Tutuila is planned in summer 2014. Objectives include gathering model calibration data, obtaining and analyzing water samples from coastal springs, defining high-level groundwater elevations, and gathering upland spring samples. In addition, student interns and research partners at American Samoa Community College will be trained to assist in sample collecting throughout 2015. Work is also continuing on the development of a recharge coverage for modeling purposes and data from new climate models is expected to be available by 2015.

A funding cut early in the project resulted in the removal of the following analyses that were stated in original proposal: 1) analysis of carbon isotopes, 2) a detailed examination of biogeochemical reactions along down gradient flow paths, and 3) the use of electrical resistivity techniques. The four primary objectives of the original proposal will remain attainable without the employment of these methods.

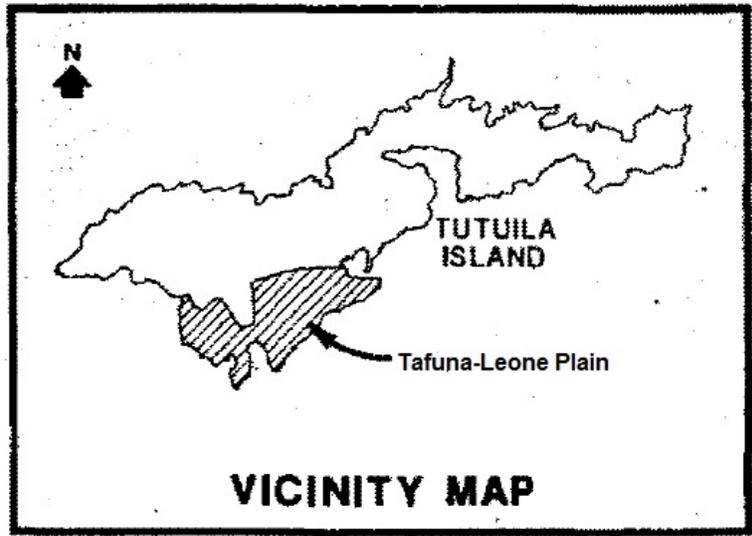


Figure 1. Map of Tutuila, American Samoa (modified from Kennedy et al. 1987).

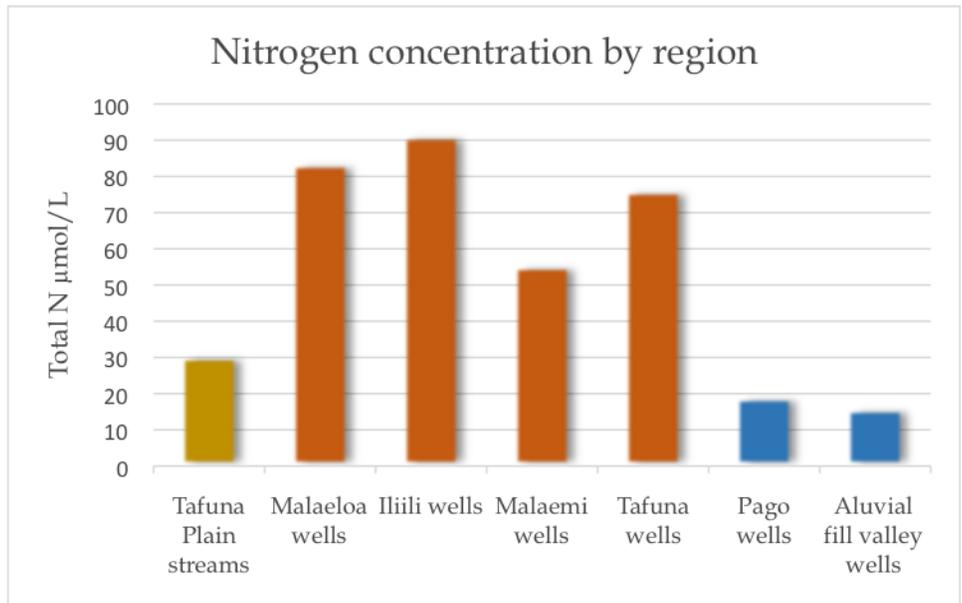


Figure 2. Average number of total N concentrations in discrete well fields and regions.

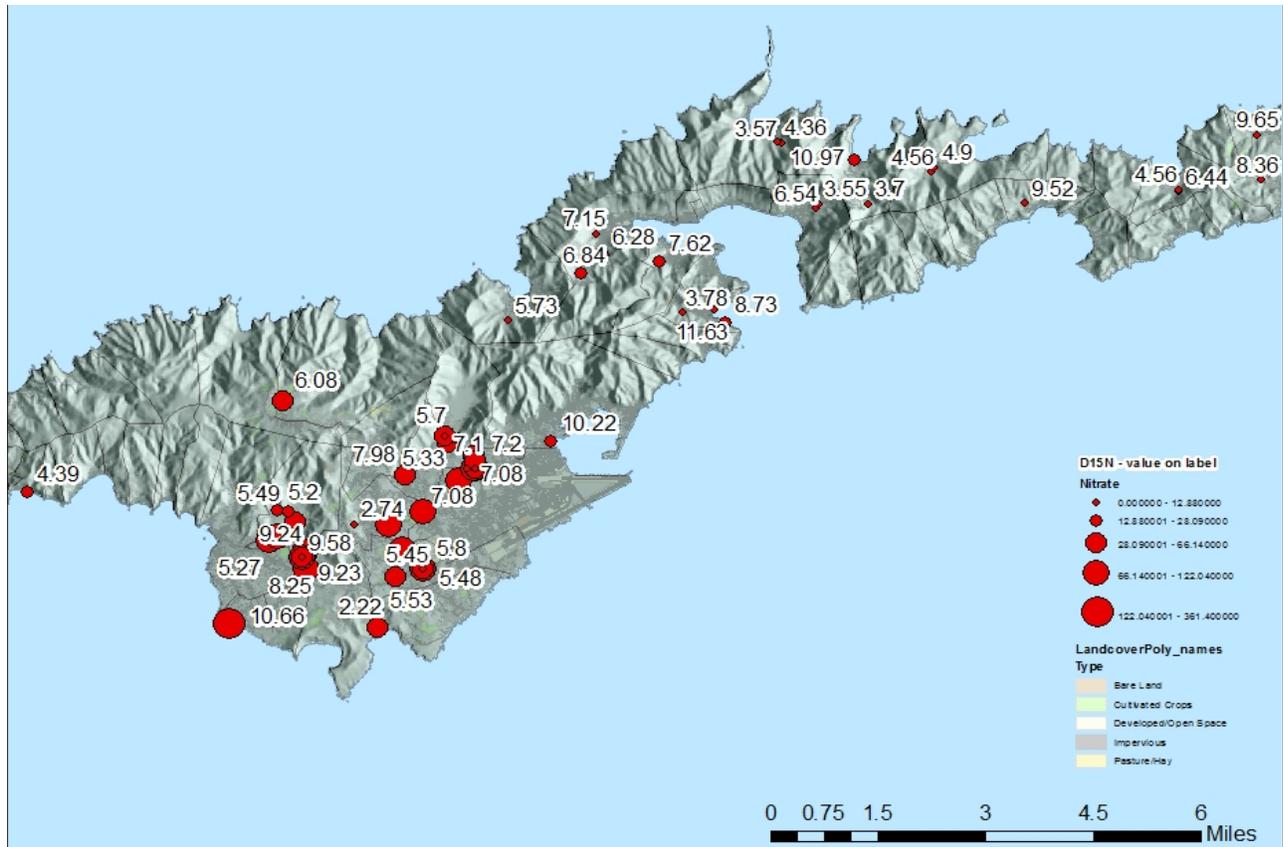


Figure 3. D15N data from water samples for wells, streams, and springs on Tutuila.

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

## Basic Information

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

## Publications

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.
2. Schubert, O.S., 2012, Changes in Vegetation and Environment over the Holocene, Ka'au Crater, O'ahu, Hawai'i, MA Thesis, Department of Geography, College of Social Sciences, University of Hawaii at Manoa, Honolulu, HI, 168 p. (in preparation, a manuscript from this research is planned for submission to Journal of Paleolimnology)

## **Problem and Research Objectives**

The climate of the Hawaiian Islands, particularly rainfall, is notoriously dynamic across geographic space (Giambelluca et al. 2013) and has long been recognized to be strongly influenced by changes in the 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 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 (Fig. 1). For this reporting period we have expanded this network to include additional sites on Molokai and Hawaii Island.

## **Methodology**

We employ two main lines of inquiry: 1) fossil pollen abundances reflecting changes in local vegetation over time (work completed during the first reporting period), and 2) stable isotope and organic geochemistry of bulk sediment and specific biomolecules (continuing through this period). Fossil pollen work and the geochemistry of Kaau Crater focused upon the last 8,000 years of well-dated sediments (Schubert 2012). Stable isotope geochemistry work for this period has focused on a comparison of two sites with contrasting rainfall, Kaala mountain (1,224 m elevation, mean annual rainfall of 2,071 mm) and high elevation Molokai (1,281 m elevation, mean annual rainfall of 3,377 mm; Giambelluca et al. 2013). We have conducted some leaf wax organic chemistry as well as the chemistry of lignin in organic sediments on Kaala and also from the Kohala Mountains on Hawaii Island. Of significance, this reporting period is comprised of new work conducted on a 9,000-year old profile from Pepeopae Bog on Molokai (a new collaboration with The Nature Conservancy on Molokai) where we have been looking at changes in lignin chemistry of plants and sediments, that is, copper oxide extractable lignin monomers measured by gas-chromatography/mass-spectrometry.

In the previous reporting period, we highlighted the water table monitoring (ongoing) being conducted at Kaau Crater. In addition to water table logging, we also have been monitoring microclimate on the Kaala and Kohala mountain on Hawaii Island. Loggers are recording air and soil temperature, relative humidity, and photosynthetically active radiation as of 2011 to 2012.

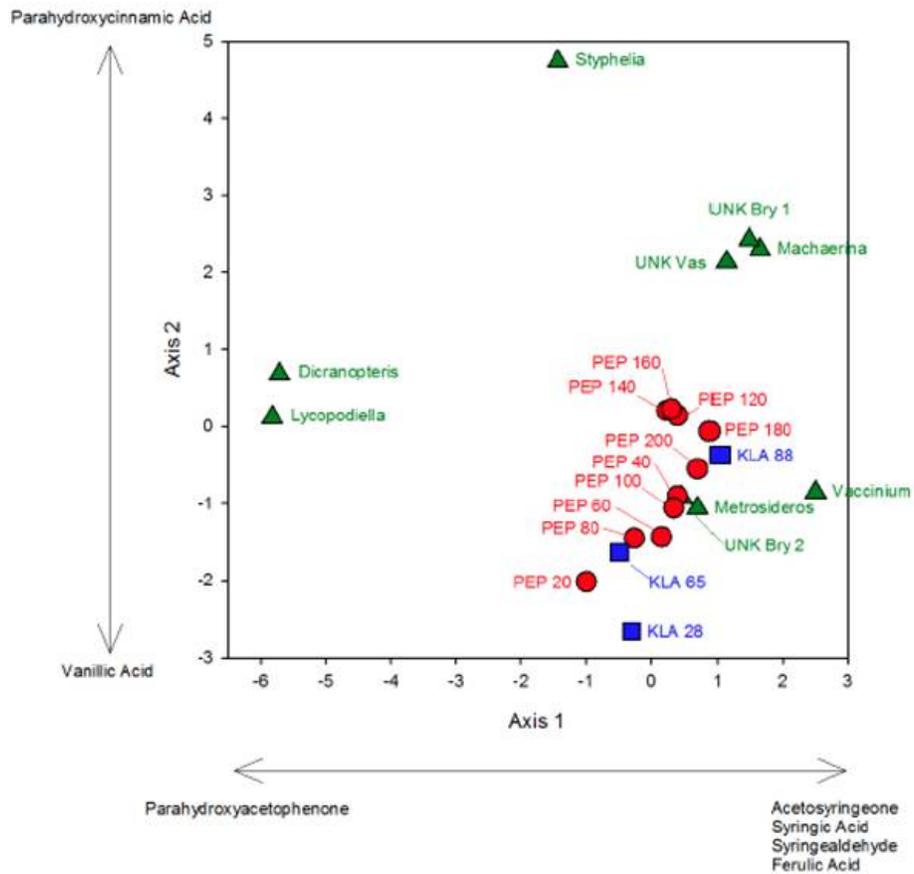
## Principle Findings and Significance

### Fossil Pollen and Stable Isotope Geochemistry

The pollen of approximately 34 different families of plants was discovered in the Kaau Crater sediments, spanning the last 8,000 years. Of interest are changes in vegetation between 5,000 and 6,000 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 Hawaii, e.g., general cessation of reef accretion at multiple high-energy windward locations across the islands (Rooney et al. 2004). Previously we measured stable isotopes in bulk sediment from Kaau Crater (previous reporting period) that supported the patterns observed in the microfossil work.

### Organic Geochemistry

In the previous reporting period, we focused on *n*-alkane sediment chemistry derived from plant leaf waxes (see previous report). In this period, we focused on using CuO- extractable lignin (Ertel and Hedges 1984) to first identify patterns among plant litter types and then to observe down-core changes in lignin monomers at the two study sites: Kaala mountain and Pepeopae Bog on Molokai. Plants and organic sediments were found to be composed of about 1 to 4% extractable lignin. We found that the dominant litter-producing plant types (lower plants such as mosses and ferns and higher plants like sedges and woody vasculars) growing at the Pepeopae site were distinct in their lignin chemistry (Fig. 1). The amount of lignin as well as the type of lignin compounds extractable from different levels of the 9,000-year old sedimentary profile at Pepeopae are shown in Figure 2. This line of inquiry is showing great promise. For example, at Pepeopae, CuO lignin chemistry has revealed a high lignin preservation between 5,000 to 10,000 years ago. Additional studies for lignin chemistry on Hawaii sediments will be conducted during the Summer of 2014 to Fall 2015.



Note: green triangles = plant litter samples, blue squares = Kaala sediment samples, red circles = Pepeopae sediment samples.

Figure 1. The Principal Components Analysis shows the results of the lignin chemistry of plants and soils. The first two principal components (Axes 1 and 2) are shown for ten CuO-extractable lignin monomers found in the leaf litter of eight dominant plant species growing at a high elevation site on Pepeopae, Molokai, as well as the lignin chemistry of fifteen organic sediment levels at Pepeopae and Oahu's Kaala mountain sites. The lignin monomers, most strongly correlated with Axis 1 and 2 PCA scores, are shown on the end of each axis where they are correlated. For example, abundances of vanillic acid are higher for samples with more negative Axis 2 scores. Collectively, Axes 1 (35%) and 2 (25%) explain more than half of the variation in the total dataset.

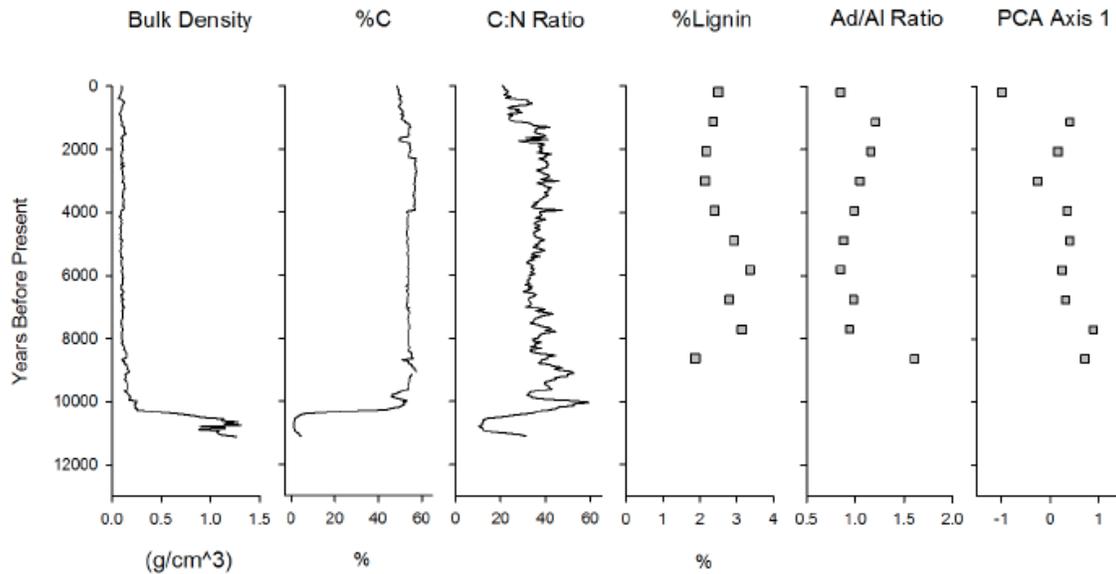


Figure 2. Results for a 10,000-year old organic sediment profile for Pepeopae Bog, Molokai: bulk density, elemental carbon, C:N<sub>org</sub>, and lignin chemistry. Lignin percentages indicate that the earlier period (between 5,000 and 10,000 years ago) contains more preserved lignin than the more recent period 5,000 years ago. The acid-to-aldehyde ratio (Ad/Al) increases when lignin is more degraded, thus the Ad/Al minimum around 6,000 years ago further supports this highly-preserved and likely wet period with greater rainfall in the early Holocene period. PCA Axis 1 scores follow Figure 1, and suggest that the early wet period had a higher abundance of sedges and woody vascular plants.

## Future Funding

Based on our findings, we received additional funding from the College of Social Sciences to further our research. These and future findings will be included in an NSF grant submission to the Paleoclimate Perspectives on Climate Change program for Fall 2014 or Fall 2015.

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## **Student Support**

Three students have been supported on this project by the Section 104 Base Grant: one Master's student (Ms. Olivia Schubert) and two undergraduate students (Mr. Rhys Ormond and Mr. Karl Hsu). Mr. Karl Hsu is a member of the University of Hawaii's Honors Program, and in the top 15% of the undergraduate class at the University of Hawaii of Manoa. Mr. Hsu also received an Undergraduate Research Grant (\$5,000) from the Undergraduate Research Opportunities Program at the University of Hawaii at Manoa. A *Notable Award* was received by Ms. Olivia Schubert at the 2011 Tester Symposium at the University of Hawaii at Manoa. She received the Best Student Poster for her presentation entitled "Long-term aspects of mountain rainfall and vegetation change, Oahu," which portrayed previous results from this project.

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

## Basic Information

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

## Publications

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.
2. There are no publications for 2013.

## **Problem and Research Objectives**

Aquaculture is an important practice in Hawaii with roots in traditional practices as well as modern technology-based solutions. Hawaii's aquaculture production rose to \$40 million in 2011 and the sector is continuing to expand; however, water quality regulations have not evolved in tandem to accommodate the needs of this growing industry. The goal of this project is to study and assess the regulatory framework related to the aquaculture industry 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 across federal, state, and county authorities. 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 based in technically based policy analyses, utilizing readily available regulatory information and scientific data through the University of Hawaii's WRRC's library as well as that of the University's Hawaii-specific scientific and regulatory history.

The study also involved 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. Literature research on comparable water quality standards and shellfish sanitation programs across the U.S. and throughout the world was also carried out. Augmented by additional funding obtained through ongoing collaboration with Sea Grant investigators (Haws 2012), we are exploring options for 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 project reviewed existing literature, followed promising developments, and consulted with a wide range of stakeholders involved in the aquaculture sector. The regulatory framework for Hawaii aquaculture has been evolving, albeit in an uncoordinated fashion. During this reporting period, a number of meetings and discussions were held with the scientific community, fishpond operators, and agency personnel to improve our understanding of the status, future prospects, and the growing research needs. The following are the identified revisions in the regulatory

framework which were detailed in the previous annual reports and further strengthened during this reporting period.

Potential revisions to the water quality standards that would support the advancement of Hawaii aquaculture include:

- (1) changing the 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.

The results of this study suggest that the State of Hawaii does not currently have sufficient resources to support the management of extensive near-shore shellfish growing grounds. However, the scenario is improving and significant accomplishments are pending that would greatly improve the regulatory framework for fishpond aquaculture. There is a great hope for the restoration of shellfish as a 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.

### **Publications Cited in Synopsis**

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](http://www.seagrant.noaa.gov/funding/2012_National_Sea_Grant_Aquaculture_Projects.pdf).

# Addressing Sewage Contamination of Nawiliwili Streams and Kalapaki Beach

## Basic Information

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

## Publication

1. There are no publications.

FINAL REPORT

**Addressing Sewage Contamination of  
Nawiliwili Streams and Kalapaki Beach**

**May 2014**

Roger Fujioka  
Marek Kirs

Project Number: 2011HI328B

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

## Introduction

EPA requires every state to use EPA approved methods to assay water samples to meet recreational water quality standards. When these water quality standards are exceeded, EPA directives conclude that the water samples are contaminated with sewage. However, studies originally conducted by WRRC in Hawaii and confirmed by research microbiologists throughout the nation, have shown that indicator bacteria 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, which are used to establish water quality standards, grow naturally in the soil environments of Hawaii (Fujioka 2001). The presence of fecal indicator bacteria in Hawaii's environmental waters can be due to soil contamination rather than sewage contamination. Additional studies by WRRC laboratories have shown that analyzing water samples for alternative microorganisms found in sewage may provide more reliable data for the presence and absence of sewage contamination.

Fujioka completed a research study on the Nawiliwili Watershed on the island of Kauai where cesspools are extensively used. The conclusions of that study are as follows: 1) Many of the microbial water monitoring data obtained from the island of Kauai were similar to data previously obtained from the island of 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 the islands of 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 of sewage pollution on the island of 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

The first objective of the research was to apply a molecular method to elucidate the source of the elevated levels of fecal indicator bacteria in the Nawiliwili watershed. Water samples will be analyzed for the human *Bacteroides* marker as well as for the alternative sewage indicator, *C. perfringens*, and coliphage. The second objective was to compare the present data with a previous study to see if there has been any major changes in the condition of the watershed.

## Methodology

A total of 117 water samples were collected by Mr. Gary Ueunten of the State of Hawaii Department of Health (HDOH), Kauai Branch, with help by Dr. Marek Kirs from ten sites in the Nawiliwili watershed at Kalapaki Beach located at the eastern section of Kauai (Fig. 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 twice a month from June to December 2011. After collection, samples were cooled on ice and shipped by air to the University of Hawaii's WRRC microbiology laboratory. All samples were analyzed within 6–9 h after sample collection for fecal indicator bacteria (enterococci, *E. coli*) and alternative fecal indicators (*C. perfringens*, coliphages). 100 ml sample volumes were filtered through membrane filters (type HA, 0.45  $\mu\text{m}$  pore diameter) and stored at  $-40^{\circ}\text{C}$  for further molecular analysis.

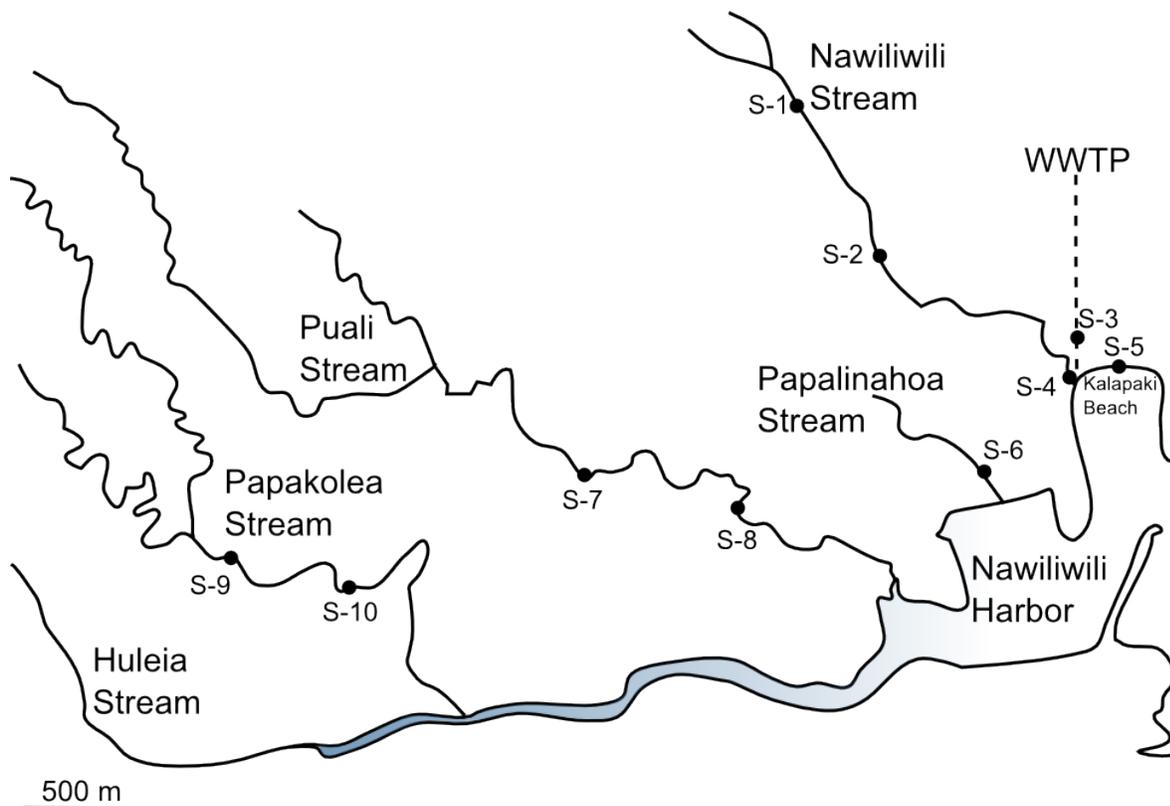


Figure 1. Nawiliwili watershed sample sites: S-1 Upper Nawiliwili Stream, S-2 Lower Nawiliwili Stream, S-3 Mariott Culvert, S-4 Pine Trees, S-5 Kalapaki Beach, S-6 Papalinahoa Stream, S-7 Upper Puali Stream, S-8 Lower Puali Stream, S-9 Upper Papakolea Stream, S-10 Lower Papakolea Stream.

Concentrations of enterococci and *E. coli* were determined using USEPA approved membrane filtration based methods 1106.1 (USEPA 2002a) and 1103.1 (USEPA 2002b). In the case of enterococci the samples were filtered through a membrane filter (Pall Corporations, Ann Arbor, MI), the filter was placed on an mE agar plate and incubated at  $42^{\circ}\text{C}$  for 48 h. After incubation filters were transferred to a pre-warmed EIA plates and incubated for additional 20 minutes.

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 membrane filter (Pall Corporations, Ann Arbor, MI), filter was placed on mTEC agar plate and incubated first at 35°C for 2 h and then in waterbath set at 44.5°C for another 22–24 h. After incubation the membranes were transferred to absorbent pad saturated with Urea Substrate Medium for 15–20 minutes. Yellow, yellow-brown and yellow-green colonies were counted as *E. coli*.

Concentrations of *C. perfringens* spores during the first sampling event were determined by incubation of 100 ml subsamples for 15 minutes at 60°C to kill the vegetative cells followed by inoculation into SFP media using Fung double tube (FDT) method (Fung et al. 2007). Due to the high background signal originating from other species of *Clostridium* in the freshwater samples, conventional mCP agar based method (Emerson and Cabelli 1982) was used for the rest of samples. After incubation at 24 h in an anaerobic chamber at 42°C for 24 h, the membranes were exposed to ammonium hydroxide fumes for 20 seconds and pink colonies were counted as *C. perfringens*.

Concentrations of somatic and male (F<sup>+</sup>) specific coliphages were identified in 5 ml sample portions using USEPA approved Method 1601 (USEPA 2001) and using *E. coli* CN-13 and *E. coli* F<sub>amp</sub> as the host, respectively. Negative samples were assayed by enrichment using 100 ml sample portions on the following day.

Concentrations of human specific molecular marker, Bacteroides (HF183), were determined using quantitative PCR (qPCR) protocols as specified in Haugland et al. (2010). A linearized plasmid containing the *Bacteroides dorei* insert was used as quantification standard. Concentration of the standard was determined on Qbit 2.0 lumionometer (Life Technologies). Quantification standard was serially diluted 10-fold from 10<sup>5</sup> to 10 copies per microliter and each concentration was seeded in triplicates in each qPCR run. Before the concentration of the human specific marker was determined, samples containing PCR inhibitors were identified using the sketa assay (Haugland et al. 2005). Reactions delayed over 3.3 threshold cycles (roughly corresponding to a 10 log underestimate) were considered inhibited and diluted 10 fold. All quantitative PCR reactions were run on a CFX96 real-time system (BioRad Laboratories Inc.). DNA was extracted from the filter concentrated environmental samples using PowerSoil DNA isolation kit (MoBio Laboratories Inc.).

Concentration of total phosphorus (PO<sub>4</sub><sup>3-</sup>) was identified using acid persulfate digestion (method 8190) on Chemical Oxygen Demand reactor (Hach, Loveland, CO). Temperature, pH, salinity and weather conditions were recorded on field by HDOH staff.

## Principal Findings and Significance

All freshwater sites, this includes all sites studied except Kalapaki Beach (which was 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 concentration of indicator bacteria were elevated, except for 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. (2011), 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 the state explicitly that no sample should exceed 89 CFU of enterococci per 100 ml (single sample maxima (SSM), HODOH 2013). This state standard was 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).

Current Hawaii recreational **marine** water quality standards are based on enterococci and state explicitly that no sample should exceed 104 CFU of enterococci per 100 ml (single sample maxima standard (SSM), HODOH 2013). Samples collected at the Kalapaki Beach did not exceed the SSM standard indicating a good water quality when the samples were collected. We did not identify any water quality issues at the Kalapaki Beach during this study.

*C. perfringens* concentrations were low during the study at all sites. Hawaii uses *C. perfringens* as a secondary sewage tracer in parallel with enterococci standard in marine and freshwaters. Warning signs are posted when 50 CFU per 100 ml limit for freshwater and 5 CFU per 100 ml limit for marine waters are coupled with enterococci standard exceedance. No freshwater 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). These data indicate that elevated concentrations of enterococci in the watershed are likely predominantly from environmental sources.

Concentrations of somatic coliphages were comparable to the results in an earlier study (geometric mean 2–289 PFU per 100 ml). The same does hold true for male (F<sup>+</sup>) specific coliphages (geometric mean 1–11 PFU per 100 ml), although concentrations were over 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<sup>+</sup>) specific coliphages. While somatic coliphages may multiply in the environment, (F<sup>+</sup>) specific coliphages cannot replicate in the environment as F<sup>+</sup> pili are not formed at or below 25°C (9) and therefore (F<sup>+</sup>) coliphages are considered better indicators of fecal contamination (animal and/or human) than somatic coliphage based on the low concentrations of (F<sup>+</sup>) specific coliphages, fecal contamination appears to be limited in the study area. It should be considered, however, that only a small percentage of humans and animals carry this group of phages, hence a leaking cesspool from a single family households or input from a small population of animals could remain undetected.

None of the 117 samples tested contained significant levels of PCR inhibitors causing more than a 10 fold underestimate of DNA concentrations. This is somewhat different from the Manoa watershed where roughly 6.8% (n=117) samples contain significant levels of PCR inhibitors (Kirs, unpublished data). This is likely due to the different water chemistry parameters, originating from different soil and land use patterns between the watersheds. It is important to

consider that DNA was extracted and purified using a spin-column based kit. While the DNA purified using this type of kit is usually free of PCR inhibitors, it is not well suited for determining absolute concentrations due to the losses during the purification process. Fortunately, when contamination source identification is needed, absolute quantifications is usually not needed and usage of this DNA extraction method is still warranted.

Four out of five (80%) R-1 water samples collected at the Lihue WWTP were positive for the human specific *Bacteroides* marker. In the positive samples marker concentration varied from 709 to 20,108 marker gene copies per 100 ml. In contrast, none of the samples collected at pristine control site (upper reaches of Lawai Stream) contained the human specific marker.

Detection of the human specific marker in the watershed samples was sporadic. The marker was detected and quantified twice at the Pine Trees (S-4) (1190–2375 gene copies per 100 ml) and once at lower Nawiliwili Stream site (S-2) (150 gene copies per 100 ml). It was also detected at the Papakolea Stream (S-9) (1058 gene copies per 100 ml).

This human specific marker has not been in used in Hawaii extensively. Therefore we also conducted a series of studies to identify prevalence and concentration of the marker in the human fecal and in raw sewage samples collected from individuals living here and wastewater treatment facilities located in Hawaii to investigate reliability of this marker in Hawaii. Roughly 80% of humans (n=10) appear to carry this marker and marker titers varied  $1.5 \times 10^4$  to  $3.5 \times 10^9$  gene copies per gram wet weight fecal matter. All collected sewage samples were positive for this marker. Marker concentration varied from  $1.55 \times 10^7$  to  $4.56 \times 10^7$  gene copies per 100 ml in the raw influent and from  $5.99 \times 10^5$  to  $6.73 \times 10^6$  gene copies per 100 ml in the effluent in the wastewater treatment facilities. Roughly two log reduction of the marker was observed within 5–6 days in marine and freshwater microcosms seeded with sewage and kept in dark. Collectively this indicates that this marker is well suited for identification of human contamination

## Summary

Analyses of 117 water samples indicate that elevated indicator bacteria concentrations in the Nawiliwili watershed appear to originate from the environmental sources. *C. perfingens* and the male specific coliphage were low at all sample sites. Human contamination was detected at several sites in the watershed, including the sites upstream from the Lihue WWTP, indicating that leaking sewer or on-site septic systems do contribute to the impairment as was suggested by earlier studies (Vithanage 2011). Based on the prevalence, concentration in sewage and human fecal samples, and decay rates, human specific *Bacteroides* marker (HF183) is well suited for source allocation in Hawaii. The water quality at the Kalapaki Beach was compliant with the State standards during the study period.

## Outcome

Within the framework of this project, Dr. Kirs was introduced to Mr. Joseph Lichwa and Mr. Philip Moravcik (both employed at WRRC, University of Hawaii at Manoa), Mr. Watson Okubo

(Section Chief, Clean Water Branch, HDOH), Mr. Ross Tanimoto (Deputy Director, Department of Environmental Services, CCH), and Mr. Ken Tenno (Laboratory Director, Laboratory Branch, Department of Environmental Services, CCH) after his arrival.

A postdoctoral researcher, Dr. Mayee Wong, was hired on January 2013 from the project funds. She was trained in all the methods used in this study. After completing her postdoctoral studies in February 2014, she was employed by the Hawaii State Department of Health where she will continue to apply skills she obtained from this study.

The project was presented at the Molecular Training Workshop at the IMS (University of North Carolina at Chapel Hill) and at the Ocean Science Meeting 2014, Hawaii that was organized by ASLO, TOS and AGU. A report based on this research is in preparation.

The study can and has been extended to other watersheds depending on the stakeholders needs. The methods and tools validated in this study can be utilized and transferred to the HDOH and CCH laboratories. Dr. Wong's current place of employment (HDOH) expresses an interest in these methods and the need for WRRC's expertise. We foresee this study to garner future proposals with NOAA and SeaGrant and to gain further support and interest from HDOH and CCH.

Table 1. Nawiliwili watershed sampling sites and their geometric means of *E. coli*, enterococci, and alternative indicators at each site.

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 <sup>+</sup> ) coliphages PFU/100 ml
S-1: Upper Nawiliwili Stream	286 (4–1560)	255 (120–480)	3 (<1–25)	32 (<1–240)	2 (<1–40)
S-2: Lower Nawiliwili Stream	1151 (640–2040)	900 (480–1880)	4 (<1–30)	108 (20–440)	8 (<1–260)
S-3: Mariott Culvert	239 (<4–7280)	780(284–2880)	4 (<1–15)	123 (20–1040)	7 (<1–160)
S-4: Pine trees	409 (<4–1440)	520 (256–1040)	4 (<1–10)	272 (80–2680)	11 (<1–260)
S-5: Kalapaki Beach	7 (<4–106)	9 (4–26)	3 (<1–15)	2 (<1–40)	1 (<1)
S-6: Papalinahoa Stream	1050 (144–7920)	1928 (1000–3640)	6 (<1–56)	289 (<1–2620)	2 (<1–500)
S-7: Upper Puali Stream	475 (92–5440)	204 (88–480)	2 (<1–25)	87 (20–800)	2 (<1–140)
S-8: Lower Puali Stream	1260 (560–3920)	1113 (332–6040)	4 (<1–15)	78 (<1–800)	1 (<1–20)
S-9: Upper Papakolea Stream	352 (40–1120)	348 (128–2040)	3 (<1–35)	214 (60–600)	11 (<1–140)
S-10: Lower Papakolea Stream	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)

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## Island Director's workshop/conference

### Basic Information

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

### Publication

1. 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.

# 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

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

## Publication

1. There are no publications.

## Problem and Research Objectives

In a consent decree, USEPA agreed to address criticisms of its current recreational water quality standards which were based on monitoring for two traditional fecal indicator bacteria or FIB (*Escherichia coli*, 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 has 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 current recreational water quality standards are based on the assumption that the source of FIB in recreational water is sewage and that the number of FIB correlates to the risk level for a sewage borne infection 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 the current recreational water quality standards do not apply. Previous studies have shown that tropical environments favor growth of FIB that accounts for the higher concentrations and persistence of *E. coli* and enterococci in environmental sites such as soil, sediments, sand, or on plants in Hawaii. An identified problem in the application of a culture based or qPCR based method to monitor *E. coli* and enterococci is that these methods measure all sources of FIB and therefore, do not distinguish between sewage sources, animal sources and environmental sources. An identified need in monitoring environmental waters in Hawaii is to correlate the concentrations of enterococci by culturable or qPCR methods with a Microbial Source Tracking assay which can confirm the presence and concentration of sewage contamination. In addition, the same water samples should be assayed for *C. perfringens* because previous data has shown that monitoring for this organism provides more reliable data for the presence of sewage than monitoring for *E. coli* or enterococci.

The second identified problem is establishing and validating the reliability of the new qPCR method for enterococci when applied to environmental water in Hawaii. WRRC will evaluate and establish the most reliable/feasible qPCR method as applied to water samples obtained in Hawaii. As part of the validation of the qPCR method, three factors that can affect the results need to be investigated. The first factor involves inhibition in the water samples to be assayed by PCR. There is evidence of more inhibition in the qPCR assay with water samples from tropical environments than those from temperate zones. In a recent study conducted at Boqueron Beach (Puerto Rico), 34% of the samples contained significant levels of PCR inhibitors. The second factor relates to how environmental sources of enterococci, as compared to sewage sources, will react to the PCR assay. The third factor relates to the expected ratio of dead to live enterococci populations in the water samples.

In summary, a trained molecular microbiologist at WRRC will evaluate and establish a reliable qPCR method for enterococci in water samples specific for the State of Hawaii. After the method has been optimized, WRRC will train the laboratory personnel of both the Hawaii State Department of Health (HDOH) and the City and County of Honolulu (CCH) in the method. Results of the study will also enable HDOH to decide if the USEPA's novel rapid test for

enterococci is suitable for Hawaii and if the test should be incorporated into the 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 WRRIP, the project is centered on 'Water Quality' and has 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, and
- 2) Establish and evaluate qPCR based MST tools in Hawaii.

## Methodology

A total of 151 monthly water samples were collected from 12 beaches on Oahu between 05/14/2013 and 04/08/2014 (Fig. 1). Beaches for this study were selected by the Clean Water Branch, HDOH. Additionally, 117 monthly water samples were collected from 9 sites in Manoa watershed between 05/21/2013 and 04/21/2014 (Fig. 1). Triplicate raw sewage, pre-UV and effluent samples have been collected from the Sand Island and Honouliuli Wastewater Treatment Plant (WWTP). Forty fecal samples were collected from cats (n=10), rats (n=10), mongoose (n=10), and human volunteers (n=10). Sample collection for this project is complete.

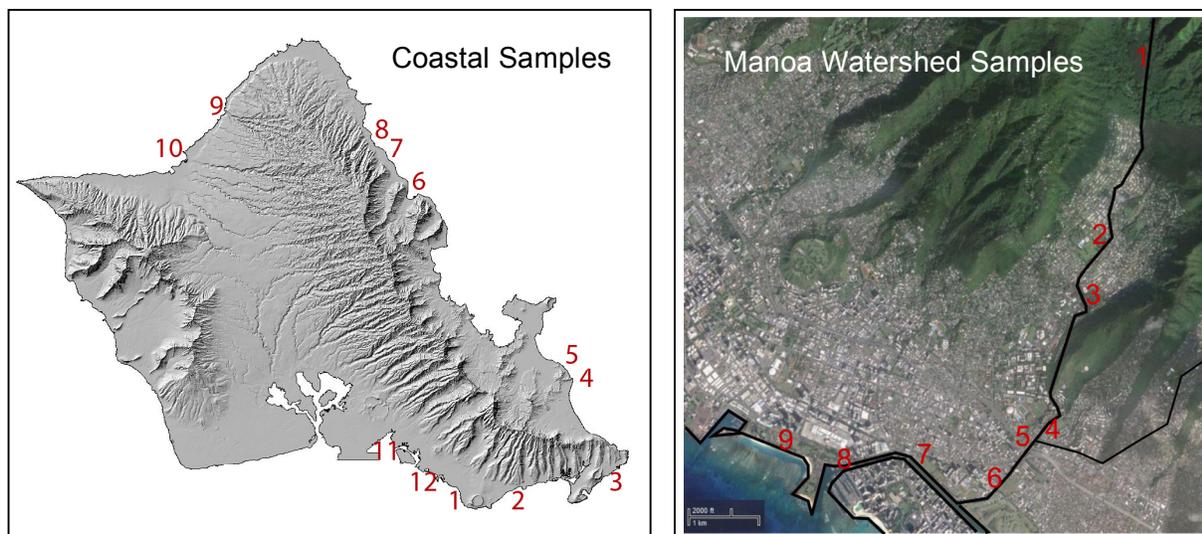


Figure 1. Sample locations.

Each water sample was analyzed for enterococci using three USEPA approved methods: Method 1600 (membrane filtration using mEI media) (USEPA 2002), Enterolert (MPN method by IDEXX Laboratories), and Method 1611 (qPCR based rapid analyses protocol) (USEPA 2012) in parallel. Analyses of samples using qPCR based Method 1611 is not complete, hence results

are not yet reported. Concentration of *C. perfringens* spores was determined using mCP media. Concentrations of F+ specific coliphages and heterotrophic plate count were determined in selected water samples only using *E. coli* Famp as a host by a single agar layer method and Simplate, respectively. Concentrations of fecal coliforms and *E. coli* were determined using Colilert-18 test kits (IDEXX Laboratories). For Method 1611, 100 ml of sample was filtered onto a polycarbonate filter (0.4 µm pore size, Whatman) and stored at -40°C until analyzed. Additionally, pH of a 300 ml sample portion was adjusted to 3.5 to facilitate binding of viruses onto filters (type GN-6, Pall Corporation). Concentrations of human specific Bacteroides marker (HF183) and human polyomaviruses (HPyV) were determined from those filters using published protocols (McQuaig et al. 2009; Haugland et al. 2010). Levels of PCR inhibitor were determined using Sketa assay as in Haugland et al. (2005).

Each fecal sample was analyzed for enterococci and *C. perfringens* as above. Coliphage concentrations and total plate count were not determined for fecal samples.

For the molecular tests, DNA was extracted using a PowerSoil DNA Extraction kit (MoBIO Laboratories Inc.). All qPCR runs included a 10-fold serially diluted 5-point standard in triplicate. A linearized plasmid containing a target specific insert was quantified on a fluorometer (Qubit 2.0 by Life technologies Inc.) and used as a standard to derive marker quantities. All samples were analyzed in duplicate.

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

## **Principal Findings and Significance**

PLEASE NOTE: This is a preliminary summary of initial findings for sampling completed on 04/21/2014. The cultivation based indicator bacteria data and molecular source tracking data are available at this stage. Analysis of the data is ongoing.

Microbial Concentrations (cultivation based tests) (tests complete, analysis ongoing)

### ***Fecal and Sewage Samples***

All human fecal samples were positive for enterococci, but only 7 out of 10 (70%) contained *C. perfringens* spores. The average concentration of enterococci in human fecal samples was  $4.07 \times 10^4$  MPN per g wet weight and ranged from  $3.1 \times 10^2$  to  $2.4 \times 10^5$  MPN per g wet weight. The average concentration of *C. perfringens* spores was  $4.52 \times 10^3$  CFU per g wet weight of feces and ranged from  $<10$  to  $3.2 \times 10^4$  CFU (Table 1). The average concentration of enterococci in cat, mongoose, and rat fecal samples was  $5.70 \times 10^5$ ,  $1.36 \times 10^6$  and  $7.07 \times 10^6$  MPN per g wet weight, respectively. Analyses and genetic typing of *C. perfringens* isolates from those animals is still in progress. The data collected so far indicated that these wild animals (cats, rats, mongoose), abundant in Hawaii urban and rural environments, may be an important source of enterococci in the environment.

Table 1. Fecal indicator bacteria concentrations in human subjects studied.

ID	Gender	Age at Date of Collection	Enterococci (MPN/g)	<i>C. perfringens</i> spores (CFU/g)
1	M	56	8.60E+02	4.10E+03
2	M	49	1.20E+04	10
3	M	45	2.40E+05	10
4	F	33	2.40E+04	9.60E+02
5	M	49	4.40E+03	<10
6	F	42	1.30E+03	150
7	F	43	4.10E+03	<10
8	M	39	1.19E+05	7.80E+03
9	M	39	6.30E+02	<10
10	F	37	3.10E+02	3.22E+04

Concentrations of enterococci in the three raw wastewater samples collected from the Sand Island and Honouliuli WWTPs averaged  $1.71 \times 10^5$  and  $5.131 \times 10^6$  CFU per 100 ml, respectively. Enterococci concentrations in the effluent were reduced to  $3.70 \times 10^3$  and  $4.55 \times 10^3$  CFU per 100 ml, respectively, indicating at least two logs reduction of indicator bacteria in both WWTPs. *C. perfringens* spores were more resistant to the treatment process. The concentration of *C. perfringens* spores in the three raw samples collected from the Sand Island and Honouliuli WWTPs averaged  $7.43 \times 10^3$  and  $1.34 \times 10^5$  CFU per 100 ml, respectively. The concentration of the spores was reduced to  $4.37 \times 10^2$  and  $2.33 \times 10^3$  CFU per 100 ml, respectively, in the effluent in both treatment plants.

### ***Manoa Watershed***

In general, based on the 117 samples collected, microbial concentrations varied vastly in the watershed. Total coliform concentrations varied from 52 to >241,960 MPN/100 ml (average 26,170 MPN/100 ml), *E. coli* concentrations varied from <10 to >241,960 MPN/100 ml (average 3,295 MPN/100 ml), enterococci concentrations varied from <10 to >241,960 MPN/100 ml (average 2,263 MPN/100 ml), *C. perfringens* spore concentrations varied from <1 to 820 CFU/100 ml (average 60 CFU/100 ml), heterotrophic bacteria concentrations varied from <2,000 to >7,388,000 MPN/100 ml, and coliphage concentrations varied from <10 to 500 PFU. Without exception, the highest concentrations of those microbial parameters were found at sites 2–6 which are located in the urbanized section of Manoa Stream.

Enterococci concentrations were elevated in all the samples collected from Manoa Stream, and exceeded Hawaii's single sample standard (Fig. 2 solid red line). Also, *C. perfringens* concentrations exceeded the 50 CFU per 100 ml limit (Fig 2. dashed pink line) at all sites except

site 1, which is located upstream from Lyons Arboretum in a non human habitated rainforest (Fig. 1). Indicator bacteria concentrations decreased at tidally influenced sites 6–8. Site 9 is a marine beach site.

Analysis of microbial data is ongoing. We are going to explore the relationship between the land use, rainfall, and simple chemical-physical parameters such as salinity and turbidity and microbial levels in this watershed.

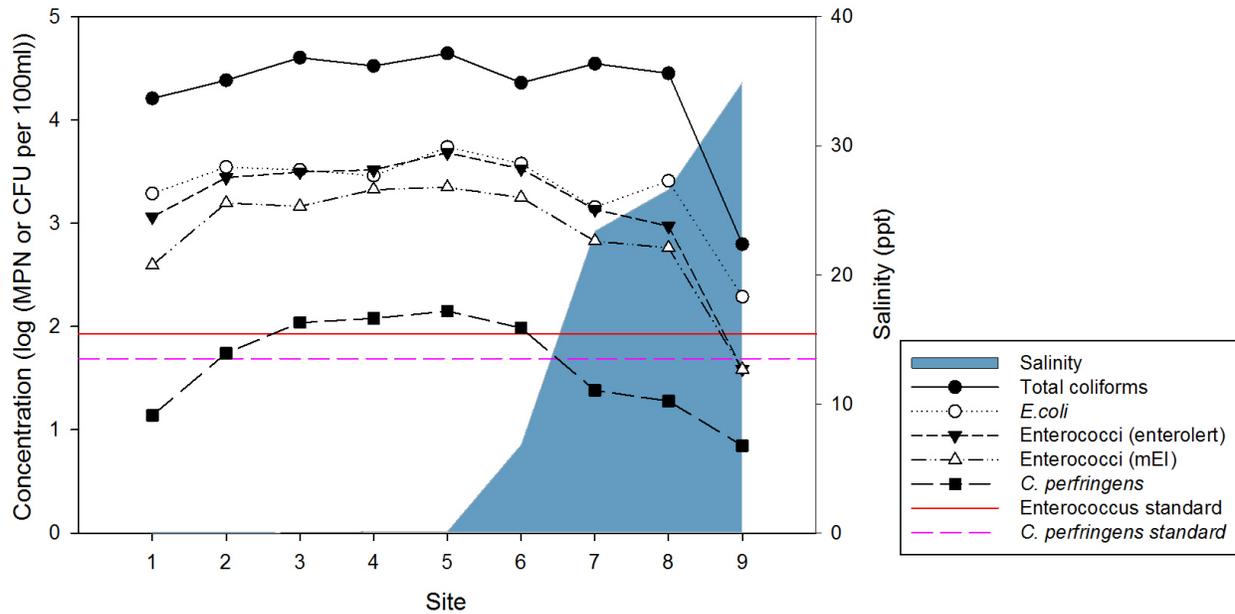


Figure 2. Distribution of fecal indicator bacteria in Manoa watershed samples (05/21/2013 and 04/21/2014, n=117).

### Coastal Samples

The State of Hawaii’s single sample maximum standard for enterococci was exceeded in 5 out of 155 samples collected (3.2% samples) at 12 beaches: twice at Keehi Lagoon and Waialae-Kahala Bay Beach [2 out of 13 samples (15%) at both sites] and once at Haleiwa Beach Park. *C. perfringens* spore concentrations exceeded the 5 CFU/100 ml limit in 4 out 5 of those samples. The exception was one of the samples collected at Waialae-Kahala Bay Beach. This suggests that human sewage is a source of impairment at those sites.

In general, enterococci concentrations varied from <10 to 389 MPN/100 ml (average 24 MPN/100 ml by Enterolert, 12 CFU/100 ml by EPA 1600 assay) and *C. perfringens* spore concentrations varied from <1 to 96 CFU/100 ml (average 3 CFU/100 ml).

## Rapid qPCR Test for Enterococci

This part of the project is ongoing. Currently over 200 PC filters are stored at  $-40^{\circ}\text{C}$  for the molecular analyses. The sample analyses were delayed to decrease the cost.

### *Correlation between EPA 1600, Enterolert, and qPCR Estimates*

As stated, the qPCR analysis is ongoing. The cultivation based data is reported below. There was good correlation between the USEPA Method 1600 and the Enterolert method [ $R^2=0.751$  Manoa watershed data;  $R^2=0.680$  coastal data (Fig. 3);  $R^2=0.855$  all data combined (Fig. 4)]. Once we have analyzed the enterococci concentrations using the qPCR based method, we can explore relationships between the results obtained using these methods and identify how beach management decisions may be affected by the choice of the method.

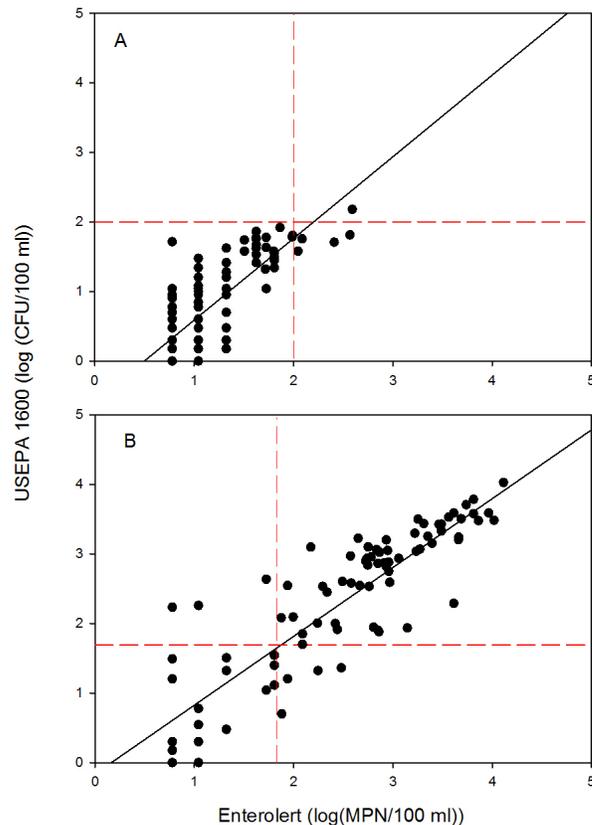


Figure 3. Relationship between enterococci concentrations (log MPN/100 ml or log CFU/100 ml) obtained by enterolert and the USEPA Method 1600 for (A) coastal samples, and (B) Manoa watershed samples. Dashed lines indicate Hawaii's single sample standard. Note: Samples exceeding the maximum quantification limit (too numerous to count) by any of the methods used were excluded from the analyses.

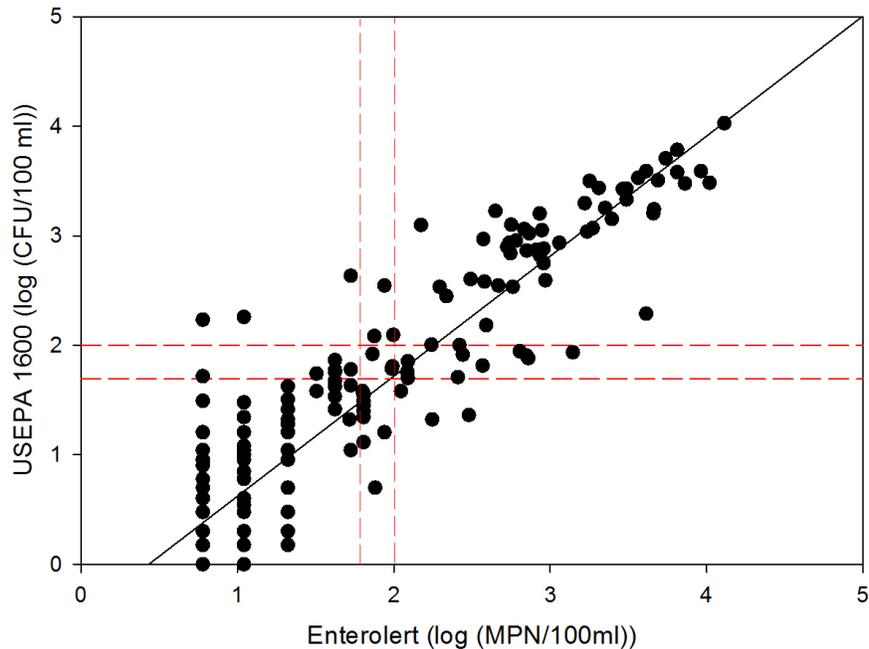


Figure 4. The relationship between the enterococci concentrations (log MPN/100 ml or log CFU/100 ml) obtained by Enterolert and the USEPA Method 1600 from pooled coastal and Manoa watershed data (n=245). The freshwater and marine enterococci single sample standards for Hawaii are indicated with dashed red lines. Note: Samples exceeding the maximum quantification limit by any of the methods used were excluded.

### ***PCR Inhibitors (ongoing)***

The level of inhibitors in the samples collected for the rapid enterococcus test has not been identified from the stored PC filters, but all samples (GN-6 filters) have been quantified for the MST markers. Therefore PCR inhibitors have been identified in the MST samples after extraction using the PowerSoil DNA extraction kit (MoBIO Laboratories, Inc.). It is important to make a distinction between the two extraction methods. USEPA's rapid protocol for enterococci involves lyses and 'cleanup' of lysed material using simple centrifugation steps. It does not involve spin-filter column based DNA purification. DNA extraction used to determine MST marker concentration (data provided below) does include a DNA purification step using spin-filter columns. While this method is known to decrease the recovery due to the additional purification steps involved, it does produce high quality DNA suitable for quantification of MST markers. This project will identify PCR inhibitors in samples extracted using both methods, but only spin-filter column based extraction method results are available and are reported below.

None of the 151 coastal samples collected contained significant levels of PCR inhibitors which would cause errors in quantification exceeding 1 log. Furthermore, no sample had a threshold cycle value shifted more than one cycle when compared to a clean water matrix. On the other hand, 6.8% of 117 samples collected from the Manoa watershed did contain inhibitors and were diluted ten-fold for the marker analyses. Therefore our data indicates that DNA extracted using

commercial kits, which include the spin column purification step, can produce high quality DNA. Unfortunately these kits may not be suitable for determining the initial marker quantities due to the losses during the purification steps. Furthermore, our data indicates that PCR inhibitors can still bias the quantification even when using a commercial kit. As a next step, the project will investigate PCR inhibitors in the samples extracted using USEPA's rapid DNA extraction protocol from material stored on PC filters (n=155 coastal samples and n=117 Manoa watershed samples).

Microbial Source Tracking (molecular tests complete, analyses ongoing)

**Marker Concentrations in the Fecal and Sewage Samples**

**Human polyomaviruses (HPyV).** None of the fecal samples was positive for the HPyV marker, but this marker was found in 100% of sewage samples tested from both WWTPs. This is not surprising as this virus is found primarily in urine because of infection by the virus of the renal tissue. The average concentration of HPyV in raw sewage varied from  $1.34 \times 10^4$  to  $2.61 \times 10^5$  and  $3.6 \times 10^2$  to  $2.72 \times 10^4$  gene copies (gc) per 100 ml in Honouliuli and Sand Island WWTP, respectively. Higher reduction of viral titres was achieved at the Honouliuli WWTP than at Sand Island WWTP. HPyV concentrations in the UV treated and chlorinated recycled (R1) water from Honouliuli WWTP ranged from below detection to 53 gc per 100 ml.

Table 2. Average microbial concentrations in treatment plants studied.

WWTP		HPyV Average Gene Copies/ 100 ml	Reduction (%)	Enterococci Average CFU/100 ml	Reduction (%)	<i>C. perfringens</i> Average CFU/100 ml	Reduction (%)
Sand Island	Influent	$2.34 \times 10^4$		$1.71 \times 10^5$		$7.43 \times 10^3$	
	After primary treatment	$2.45 \times 10^4$	-4.7	$2.67 \times 10^4$	84.4	$1.53 \times 10^3$	79.4
	UV treated effluent	$1.34 \times 10^4$	42.7	$3.70 \times 10^3$	97.8	$4.37 \times 10^2$	94.1
Honouliuli	Influent	$1.76 \times 10^5$		$2.43 \times 10^6$		$1.34 \times 10^5$	
	After primary treatment	$1.10 \times 10^5$	37.6	$4.03 \times 10^5$	83.4	$4.90 \times 10^4$	63.4
	After secondary treatment	$4.88 \times 10^3$	97.0	$4.55 \times 10^3$	99.8	$2.33 \times 10^3$	98.3

**Human Bacteroides marker (HF183).** The human Bacteroides marker was detected in 80% of the human samples tested. Marker concentration varied between  $1.5 \times 10^4$  and  $3.5 \times 10^9$  gc wet weight of human fecal matter. All collected sewage samples were positive for this marker. Marker concentration varied from  $1.55 \times 10^7$  to  $4.56 \times 10^7$  gc per 100 ml in the raw influent and from  $5.99 \times 10^5$  to  $6.73 \times 10^6$  gc per 100 ml in the effluent. Roughly two log reduction of the

marker was observed within 5–6 days in marine and freshwater microcosms that were kept in dark.

This marker was identified in a single cat (10%) and in four (40%) mongoose samples, but the concentration was  $9.56 \times 10^3$  gc per g of wet weight in cat feces and varied between  $2.1 \times 10^2$  and  $1.05 \times 10^4$  per g of wet weight in mongoose feces. These concentrations are at least 4 logs less than the average human *Bacteroides* marker concentration in human fecal samples ( $2.51 \times 10^8$  gc per g).

### Marker Decay Rates

Decay rates of both markers were comparable in freshwater and seawater microcosms (Fig. 4). Human polyomavirus concentration decreased two logs (100%) by day 19 and the human *Bacteroides* by day 6, indicating faster decay rate of the bacterial marker. The decay rate of the human *Bacteroides* marker, was almost twice as fast as cultivable enterococci (2 log reduction of molecular signal by day 11 in both environments) while human polyomavirus decay was roughly two times slower than cultivable enterococci. No significant decrease of *C. perfringens* spore numbers was observed within 40 days. This decay experiment indicates that a molecular marker signal can decay relatively quickly in the environment, although a small fraction of human *Bacteroides* residue was detectable 30 days after the start of the experiment. The decay is likely more rapid in the environment due to the UV exposure, grazing, etc.

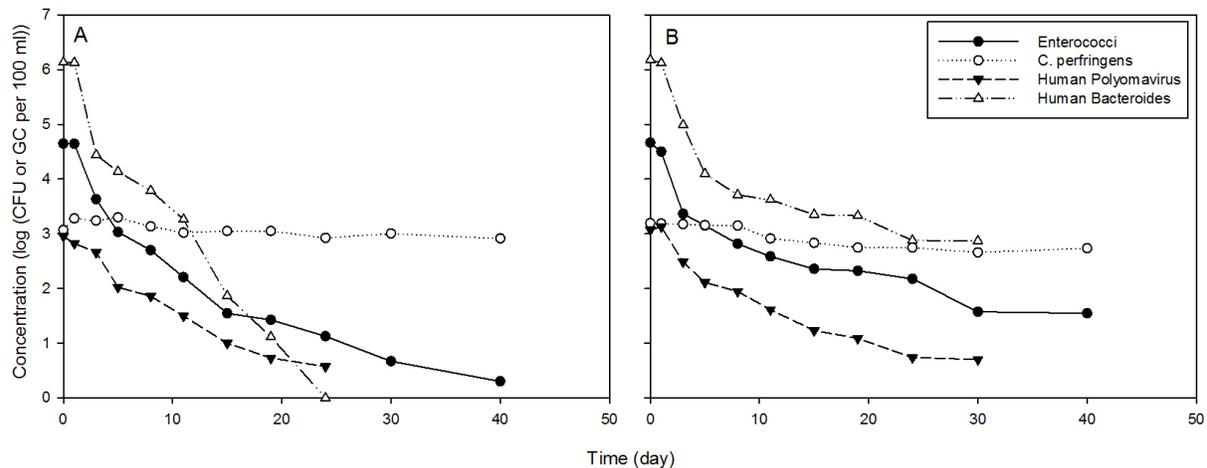


Figure 5. Decay of indicator bacteria in (A) Manoa Stream water and (B) Ala Moana beach water based on triplicate experiments. Triplicate microcosms were kept at room temperature in the dark. Water was filter sterilized then seeded with sewage at time 0.

### Manoa Watershed

The Human polyomavirus and human *Bacteroides* markers were identified at sites 2–9. Human *Bacteroides* marker was also identified at site 1 (non human habitated rainforest site), but only on

two occasions (Table 1, Fig. 6). Furthermore, the human *Bacteroides* marker concentrations were 2 logs lower at site 1 than those at sites in the urbanized area (Figs. 6 and 7). It is likely that the signal originated from cross-reactivity that was discussed in an earlier section. It is unlikely that human sewage is present at that site deep in the rainforest. This conclusion is also supported by the *C. perfringens* concentrations at this site (ranging from <1 to 40, average 11 CFU/100 ml) which never reached the 50 CFU per 100 ml that is typically associated with sewage impacted freshwater systems. Nevertheless, our source specific markers combined with traditional microbial data strongly suggest sewage based impairment in the urbanized section of the watershed (sites 2–8). The sewage signal appears diluted in the tidally influenced sections (sites 6–9).

Table 3. The number and percentage of positives for the human polyomavirus and the human *Bacteroides* marker at each site in the Manoa watershed. (n=13 at each site.)

Site:	1	2	3	4	5	6	7	8	9
<i>Bacteroides</i>	2 (15%)	13 (100%)	12 (92%)	11 (85%)	12 (92%)	11 (85%)	9 (69%)	10 (77%)	5 (38%)
Polyomavirus	0 (0%)	5 (38%)	8 (62%)	8 (62%)	7 (54%)	6 (46%)	0 (0%)	1 (8%)	3 (23%)

Note: Site 1 = non human habitated rainforest, sites 2–8 = urbanized area, and sites 6–9 = tidally influenced.

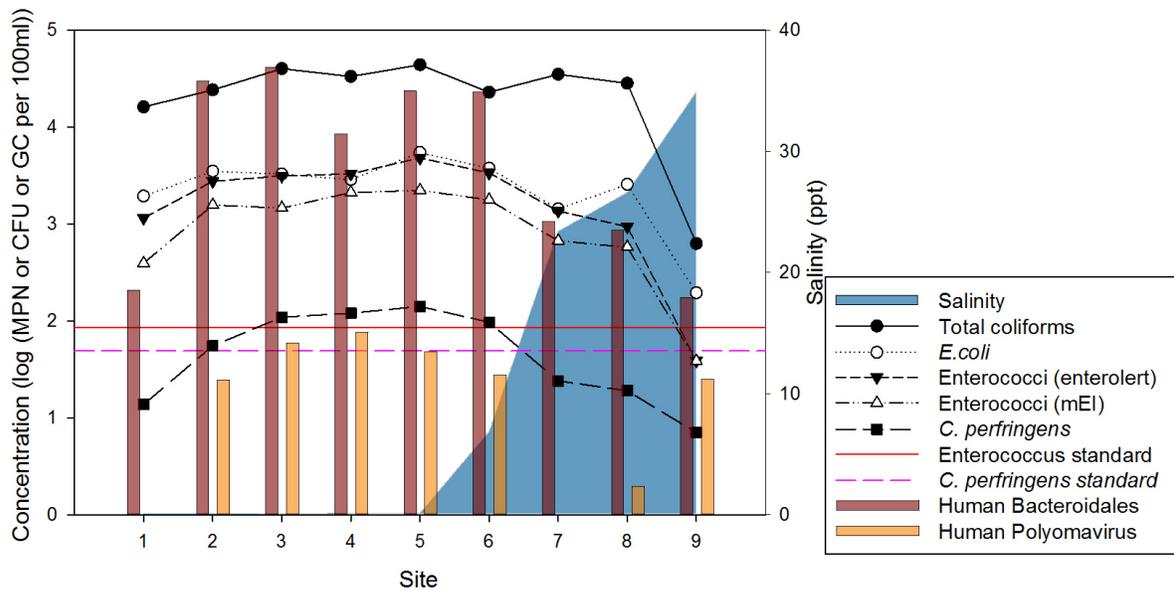


Figure 6. Indicator bacteria and molecular marker concentrations in the Manoa watershed.

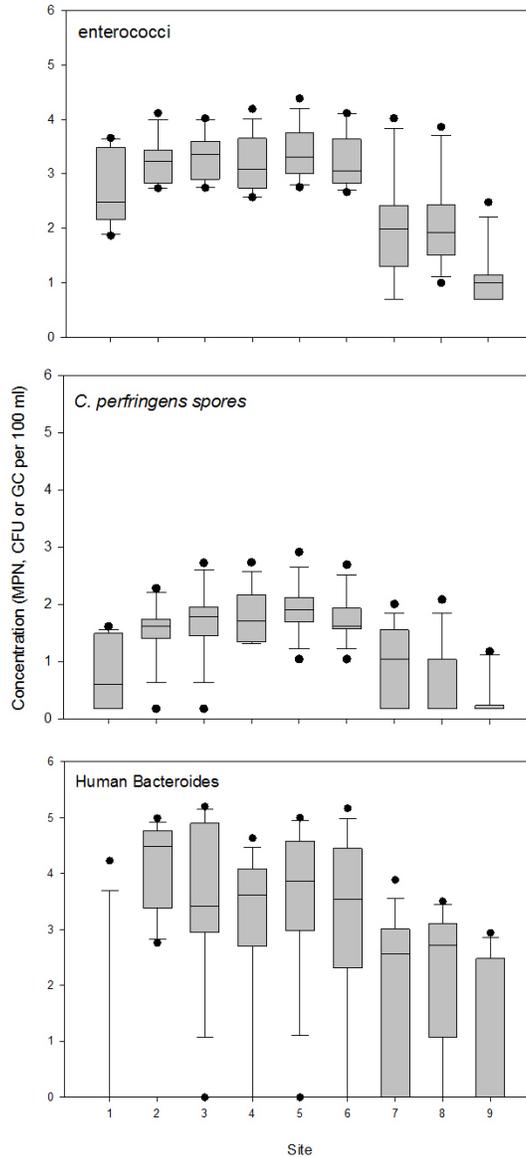


Figure 7. Indicator bacteria concentrations and human Bacteroides concentrations in the Manoa watershed.

### *Coastal Samples*

The human Polyomavirus and human Bacteroides markers have been identified only in a limited number of coastal samples. Human polyomavirus was identified twice at San Souci, and once at Kahala and Punaluu beaches. None of the samples positive for human polyomavirus contained elevated levels of indicator bacteria. The human Bacteroides marker was detected in 4 samples collected at Keehi Lagoon, three samples from the Waimea Bay shoreline and Haleiwa Beach park, and twice from Kahala, San Souci and Kailua. There was no apparent correlation between indicator bacteria levels and source specific markers. The analyses are ongoing.

## Project Output

Opportunities to extend this study to other islands and watersheds are being discussed with the HDOH. Methods and tools validated in this study could be used and can be transferred to our partner laboratories at DOH and CCH as needed.

The project was presented at the Molecular Training Workshop at the IMS (University of North Carolina at Chapel Hill) as well as at the Ocean Science Meeting 2014 organized by ASLO, TOS and AGU. Two manuscripts from this project are in preparation.

## Publication Cited in Synopsis

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- Haugland R.A., S.C. Siefring, L.J. Wymer, K.P. Brenner, and A.P. Dufour, 2005, Comparison of enterococcus measurements in freshwater at two recreational beaches by quantitative polymerase chain reaction and membrane filter culture analysis, *Water Research*, 39(4), 559–68.
- McQuaig S.M., T.M. Scott, J.O. Lukasik, J.H. Paul, and V.J. Harwood, 2009 June, Quantification of human polyomaviruses, JC virus, and BK virus by TaqMan quantitative PCR and comparison to other water quality indicators in water and fecal samples, *Applied and Environmental Microbiology*, 75(11), 3379–88.
- USEPA, 2002, Method 1600, Enterococci in water by membrane filtration using membrane-enterococcus Indoxyl-D-Glucoside agar (mEI), U.S.E.P.A, Office of Water, Washington DC, Washington, 14 p.
- USEPA, 2012, Method 1611, Enterococci in water by TaqMan® quantitative polymerase chain reaction (qPCR) assay, Office of Water, Washington DC, Washington, 37 p.

# Promoting Water Sustainability Literacy

## Basic Information

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

## Publication

1. There are no publications.

## **Methodology and Principal Findings**

The project provided faculty and students opportunities for building sustainability literacy through civic engagement. We encouraged students to confront water challenges by developing, articulating, and disseminating knowledge through collaboration with UHM faculty and fellow students, as well as government agencies involved in water management. Students were involved in planning events, including the University of Hawaii Sustainability Summit and Earth Day 2014, and presented conference posters and exhibit materials in collaboration with WRRC faculty and affiliates.

We were able to utilize campus facilities and grounds as a sustainability training and resources center, and to guide students to aspects of the university's Physical Plant that provided relevant graduate and undergraduate research opportunities. Students learned to recognize the need for input from a variety of disciplines and stakeholder groups in solving environmental problems and became conversant in water-related fields of study from across the academic continuum. Students expanded their understanding of water issues both on and off campus, and enhanced their sustainability literacy, a qualification that is increasingly demanded by decision makers and environmental managers across all job markets.

## **Significance**

Students in the Fall 2013 UHM Environmental Studies Practicum taught by WRRC faculty member John Cusick completed several internship and campus action projects that supported project objectives. Building campus water sustainability literacy nurtured greater capacity for critical thinking, problem solving, individual responsibility, and contributed to achieving the UHM Strategic Plan goal of developing a sustainability ethic on campus. In addition to the UHM Chancellor declaring Spring 2012 as a "Semester of Sustainability", the UH Board of Regents agreed to a System-wide sustainability policy in Spring 2014. Students were instrumental in presenting testimony and advocating for this policy achievement. In support of water literacy project efforts, the UHM Ecology Club hosted 2014 Earth Week festivities on campus on April 24 where dozens of informational booths were set up in the Sustainability Courtyard for campus and community organizations. Several hundred people, including the UHM Chancellor and the Mayor of Honolulu, attended the event. Environmental author, founder of 350.org, and Middlebury College Scholar in Residence Bill McKibben gave a keynote address to an estimated audience of 500 that evening as part of the UHM Dai Ho Chun Distinguished Lecture Series.

**University of Hawaii at Manoa Fall 2013 Water Sustainability Literacy Internships and Campus Action Projects**

<b>Project</b>	<b>Sponsor</b>	<b>Outputs</b>
Environmental Center Adopt-A-Landscape	UHM Facilities Maintenance Office	Realigned garden beds, installed rain catchment system, irrigation lines and timer, selected and planted replacement vegetation
Community Outreach and Education Program	Malama Maunaloa	Planned events, recruited volunteers, networked with the business community
Community Outreach and Education Program	Hui O Koolaupoko	Planned and promoted watershed protection events, recruited volunteers
Bellows Beach Dune Restoration	U.S. Air Force Civil Engineer Center	Reestablished native for flora and fauna through beach dune outplantings

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

## Basic Information

<b>Title:</b>	Fate and Transport of Pharmaceutically Active Compounds in Simulated Bank Filtration System
<b>Project Number:</b>	2012HI362B
<b>Start Date:</b>	3/1/2012
<b>End Date:</b>	2/28/2014
<b>Funding Source:</b>	104B
<b>Congressional District:</b>	Hawaii 1st
<b>Research Category:</b>	Ground-water Flow and Transport
<b>Focus Category:</b>	Water Supply, Water Quality, Geochemical Processes
<b>Descriptors:</b>	
<b>Principal Investigators:</b>	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)
4. 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.
5. D'Alessio, M., and C. Ray, 2013, "Environmental fate of selected PhACs during RBF." (in preparation for submission to Water Research, July 2013)
6. 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)
7. D'Alessio, M., and C. Ray, Fate and transport of selected pharmaceutically active compounds during simulated riverbank filtration: Impact of redox conditions, temperature and level of organics. (in preparation)
8. D'Alessio, M., and C. Ray, Impact of infiltrated air on the dynamic of redox conditions as well as on the removal of pharmaceutically active compounds in simulated riverbank filtration during clogging conditions. (in preparation)

FINAL REPORT

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

**May 2014**

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Project Number. 2012HI362B

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## **Abstract**

The occurrence of pharmaceutically active compounds (PhACs) in environmental waters has drastically increased during the past few decades. Riverbank filtration (RBF) may be a possible method to remove PhACs. The objectives of this study were to investigate the impact of oxygen, the organic matter content of the source water, and seasonal variability of temperature on the removal of selected PhACs; and the occurrence of air below the clogging layer on the dynamics of redox conditions. The behavior of six PhACs (caffeine, carbamazepine, 17- $\beta$  estradiol [E2], estrone [E1], gemfibrozil, and phenazone) was evaluated by using column experiments and by using a sandbox during simulated RBF.

RBF can be used to remove some PhACs found in surface waters. The geochemistry of the RBF site is expected to play a key role in their removal. Depending on the compound, removal may occur due to biodegradation and/or adsorption with environmental variables such as oxygen or temperature affecting the removal process.

Results from this study showed that the limited removal of carbamazepine (<20%) and gemfibrozil (<30%) was not affected by the different environmental conditions. Removal of phenazone occurred only under aerobic conditions, while removal of caffeine was highly impacted by the level of organics as well as by the temperature. Like caffeine, removal of E2 was affected by the level of organics in the feed water. Slower removal of PhACs occurred during winter conditions. The study also demonstrated that the presence of air beneath the riverbed can enhance the development of locally present aerobic conditions which may lead to an enhanced removal of redox sensitive PhACs.

## **Problem and Research Objectives**

Riverbank filtration (RBF) represents a natural filtration that has been used to provide drinking water to communities for more than a century in Europe and for half a century in the United States (Ray et al. 2002). RBF provides about 50% of the potable water in the Slovak Republic, 45% in Hungary, 16% in Germany and 5% in The Netherlands (Dash et al. 2008). RBF facilities are also located on the USA mainland (i.e., Kentucky, Nebraska, and California). Many other countries around the world including India, China, Korea, Jordan, and Egypt recently have started to evaluate the feasibility of using RBF for water treatment (Ray 2008; Ray and Shamrukh 2011). RBF systems consist of a series of abstraction wells in the vicinity of a stream or lake resulting in groundwater depletion that forces river or lake water to infiltrate into the subsurface towards the abstraction wells (Hoppe-Jones et al. 2010). RBF wells can be either vertical or radial (like collector wells). The application of one type versus the other is related to the characteristics of the aquifer (Ray et al. 2002). The advantages of RBF compared to other water technologies are related to the low capital investments and low operating costs, as well as the ability to remove suspended particles, biodegradable compounds, bacteria, and viruses (Ray et al. 2002). RBF can also buffer possible fluctuations in the quality of the water produced (Ray et al. 2002). The two major limitations to the effectiveness of the RBF are the development of an unsaturated zone (Su et al. 2007) near the collector wells and riverbed clogging (Schubert 2002). Other possible limitations of RBF are related to increases in hardness, ammonium, dissolved iron

and manganese, and the possible formation of malodorous sulfur compounds due to changing redox conditions (Ray et al. 2002; Schubert 2002).

During the last few decades, the presence of trace organic chemicals (TrOCs), such as personal care products (PCPs), household chemicals, and pharmaceutically active compounds (PhACs) dramatically increased in the environment (Ternes 1998; Kolpin et al. 2002; Snyder et al. 2007; Benotti et al. 2009). The presence of these chemicals has been reported in surface water and groundwater in the United States (Kolpin et al. 2002; Benotti et al. 2009) and in Europe (Ternes, 1998). Conventional wastewater treatments are not adequate to fully remove certain groups of TrOCs that can then be discharged into rivers (Heberer 2002; Yu et al. 2006). Some of these chemicals, even at concentrations of a few ng/L may have carcinogenic effects on the aquatic and terrestrial organisms (Thiele-Bruhn and Beck 2005; Vajda et al. 2008; Madureira et al. 2011) and potential adverse effects on human health (Boxall 2004; Fent et al. 2006). There are still uncertainties regarding the environmental fate of these micropollutants.

The behavior of PhACs at bank filtration sites is impacted by the physico-chemical properties of the compounds, and by the local conditions, such as redox conditions, temperature and travel time (Grünheid et al. 2005; Massmann et al. 2006; Heberer et al. 2008; Hoppe-Jones et al. 2010).

The objectives of this study were to investigate the role of a) the oxygen level— aerobic vs. anaerobic; b) variable organic matter content of the source water; c) seasonal variability of temperature—summer vs. winter; and d) air present below the clogging layer on the removal of selected PhACs and the dynamics of redox conditions during simulated RBF.

## **Methodology**

The PhACs used in this study were selected according to their pharmaceutical class, occurrence in the environment and public interest, their toxicity, their environmental fate, their behavior under different redox conditions, and the availability of analytical standards and adequate instrumentation. Caffeine, carbamazepine, estrone (E1), 17- $\beta$  estradiol (E2), gemfibrozil, and phenazone were selected.

The fate and transport of the six selected PhACs were investigated using columns and a sandbox (a simulation of RBF). Columns were used to evaluate the impact of temperature, redox condition and organic content on the fate and transport of the selected PhACs. The sandbox was used to evaluate the impact of clogging on the dynamics of the redox conditions as well as the removal of selected PhACs by RBF.

The column experiments used flow-through stainless steel columns, 4.75 cm internal diameter, and 14.50 cm long. Light-excluding stainless steel columns were selected to minimize the effect of photo-degradation on the selected PhACs as well as the potential adsorption of these compounds onto the internal wall of the column. The columns were operated under a recirculating flow regime. The same water passed repeatedly through the column and the inflow reservoirs also served as outflow containers. A four channel head peristaltic pump (Cole-Parmer, Vernon Hills, IL, USA) was used to simultaneously introduce the different feed solutions to the

different column setups at the same hydraulic loading rate of 0.2 mL/min. Samples were collected daily from each reservoir to evaluate the levels of the different PhACs. The silica sand (#16 to #30 sieve) used in the column was pre-acclimated according to the different environmental conditions— aerobic and anaerobic, summer and winter, with and without humic acid (HA)—for approximately three months. During this period the TOC/DOC removal by the sand in the columns reached a plateau. Once this plateau condition was achieved the selected PhACs were added. An abiotic (sterile) control was used to evaluate any possible sorption of the selected PhACs to the column packing material. The abiotic control was run only under summer conditions. No abiotic control was used during the simulated winter conditions because low temperature usually limits microbial activity.

A sandbox consisting of two side-by-side transparent plexiglass rectangular boxes each with a width, height, and depth of 140×30×10 cm was used to simulate RBF. The entire unit was covered to prevent algal growth. The sandbox was dry-packed with small increments of silica sand (#16 to #30 sieve) and manually compacted during the packing process. Five cm of pea gravel were placed at the bottom of the sandbox to support the sand. The outflow from each side of the sandbox was constantly monitored throughout the study by using two automated balances connected to a datalogger (Campbell Scientific, Logan, UT). Oxygen probes (Ocean Optics, Dunedin, FA) and redox probes (HYPNOS III, The Netherlands) on each box allowed for in site measurements. Three oxygen probes were located at 10, 50, and 105 cm below the water-sand interface on each side of the sandbox. Four redox probes were located 5, 15, 50, and 105 cm below the water-sand interface. Seven stainless steel sampling ports were located at 10, 15, 20, 40, 85, 135, and 140 cm, below the water-sand interface. The number of probes and sampling ports was higher in the top section of the sandbox to monitor the change in oxygen levels and PhACs occurring just beneath the biological layer or *Schmutzdecke* formed on the top surface of the sand. A dual-head peristaltic pump (Cole-Parmer, East Bunker Court Vernon Hills, IL) was used to simultaneously introduce the feed water to the two sides of the sandbox under down-flow conditions (feed water was not recirculated).

The experimental conditions selected for use with the sandbox were chosen based on the results from the column experiments. From the different combinations of oxygen, level of organic and temperature used in the column experiments, aerobic, summer and two levels of organics (TOC = 3 mg/L and 20 mg/L) were selected for the sandbox. For 16 days, both sides of the sandbox were conditioned with water collected from Lake Wilson and filtered through 11 µm filter paper (GE Healthcare Bio-Sciences, Pittsburgh, PA); after that one side of the sandbox, referred to as LW, received unfiltered lake water alone (TOC = 3 mg/L), while the other side, referred to as LW-HA, received unfiltered lake water with HA (TOC = 20 mg/L). On day 21, a mixture of six PhACs was added to the feed water of each side (referred to as a spike) to evaluate the ability of the sandbox system to remove the selected PhACs, and the impact of organic matter (HA) on the removal of the PhACs. The addition of PhACs continued for approximately 30 days, after which the injection of PhACs was suspended for approximately 60 days. During this period of no PhAC input, LW received lake water alone, while LW-HA received lake water with HA.

The particles present in the feed water caused a clogging layer to develop at the water-sand interface in LW and LW-HA that led to a reduction in the outflow. Prior to the start of the second spike, the outflow was increased to match the outflow that occurred during the first spike, and air

was introduced below the sand-water interface by placing multiple pipettes (open to the atmosphere) in the top 2 cm of the packing material. The introduced air led to the presence of locally aerobic areas. This new configuration was established to simulate the high pumping rate that occurs in the presence of a developing clog at a RBF site, and the possible entry of air from the bank. These conditions were observed at two riverbank filtration facilities: Sonoma County Water Agency (Su et al. 2007) and Louisville Water Company (personal communication). After the presence of air below the water-sand interface was established in both sides of the sandbox, a second spike of 6 PhACs was conducted for approximately 30 days.

## Principal Findings and Significance of the Column Experiments

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

1. Those PhACs persistent under different environmental conditions: carbamazepine (Fig. 1) and gemfibrozil; and
2. PhACs removed only in the presence of specific conditions: phenazone (Fig. 2), caffeine (Fig. 3), E2 (Fig. 4), and E1 (Fig. 5).

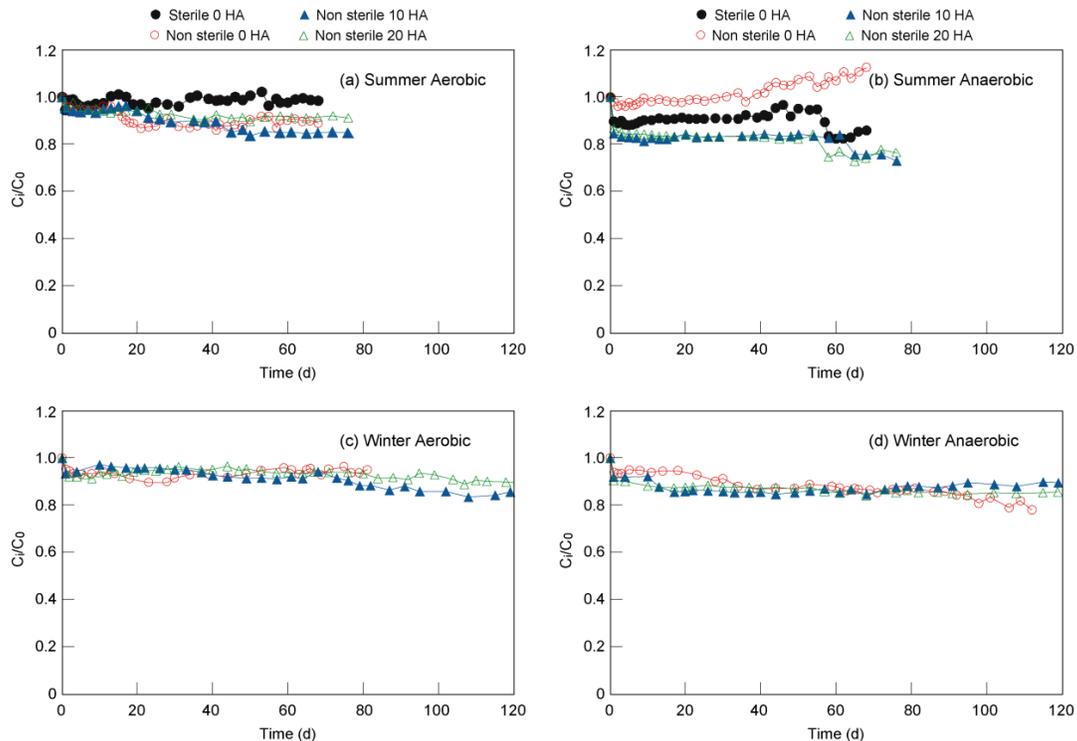


Figure 1. Break through curves of carbamazepine during (a) summer and aerobic, (b) summer and anaerobic, (c) winter and aerobic, and (d) winter and anaerobic conditions.

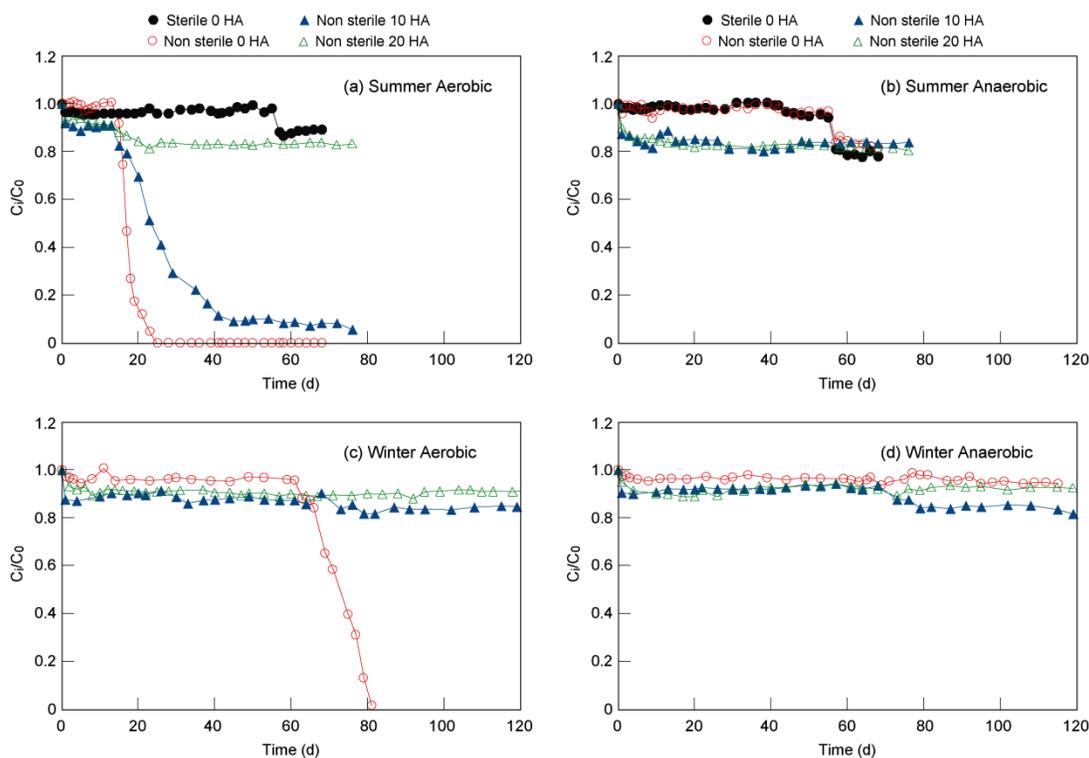


Figure 2. Break through curves of phenazone during (a) summer and aerobic, (b) summer and anaerobic, (c) winter and aerobic, and (d) winter and anaerobic conditions.

## PhACs Persistent Under Different Environmental Conditions

Carbamazepine (Fig. 1) and gemfibrozil were persistent under aerobic and anaerobic conditions as well as during summer and winter. Only limited removal of <20% was observed for carbamazepine and <30% for gemfibrozil was observed.

## PhACs Persistent Under Different Environmental Conditions

Phenazone was highly impacted by the different environmental conditions in the following order: level of oxygen > level of HA > temperature (Fig. 2). Phenazone was completely removed only under aerobic conditions, and in the presence of a low to moderate levels of HA (TOC <10 mg/L). Complete removal of phenazone occurred within 22 days during the summer and approximately 80 days during the winter. These results suggested that a shorter acclimation time, 20 days during the summer as opposed to 70 days during the winter, was needed to develop the microbial population able to degrade phenazone. Faster rate of removal (4×) occurred during the summer than during the winter.

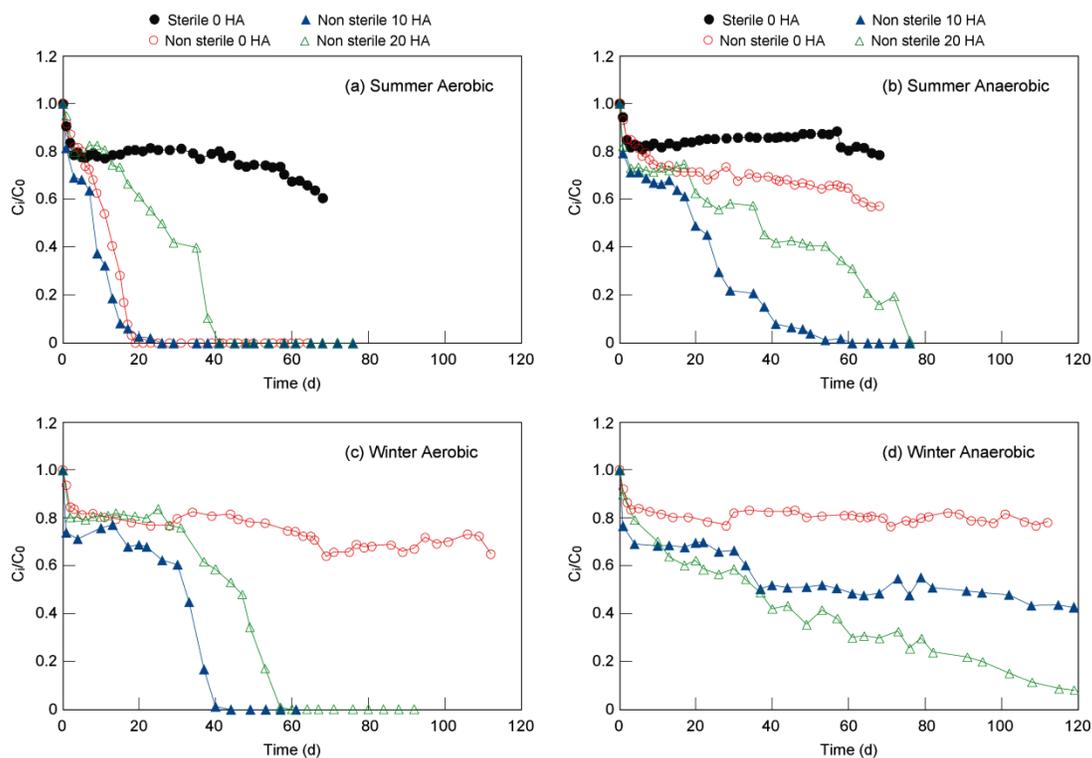


Figure 3. Break through curves of caffeine during (a) summer and aerobic, (b) summer and anaerobic, (c) winter and aerobic, and (d) winter and anaerobic conditions.

Caffeine was also impacted by the different environmental conditions in the following order: level of HA > temperature > level of oxygen (Fig. 3). In the presence of lake water alone (no HA) caffeine was completely removed only during the summer under aerobic condition. On the other hand, in the presence of HA, caffeine was completely removed regardless of the presence of oxygen or the temperature. Slower removal occurred during the winter. In lake water alone biodegradation was the main mechanism of caffeine removal, but in lake water with HA, adsorption was the primary mechanism of removal.

A decrease in caffeine was observed at the beginning of each break through curve including the sterile control suggesting the occurrence of sorption of caffeine to the packing material (Figs. 3 a–b). However, progressive removal under non sterile conditions and no further removal under sterile conditions suggested that biodegradation represented the predominant removal mechanism involved in lake water without HA (Fig. 3).

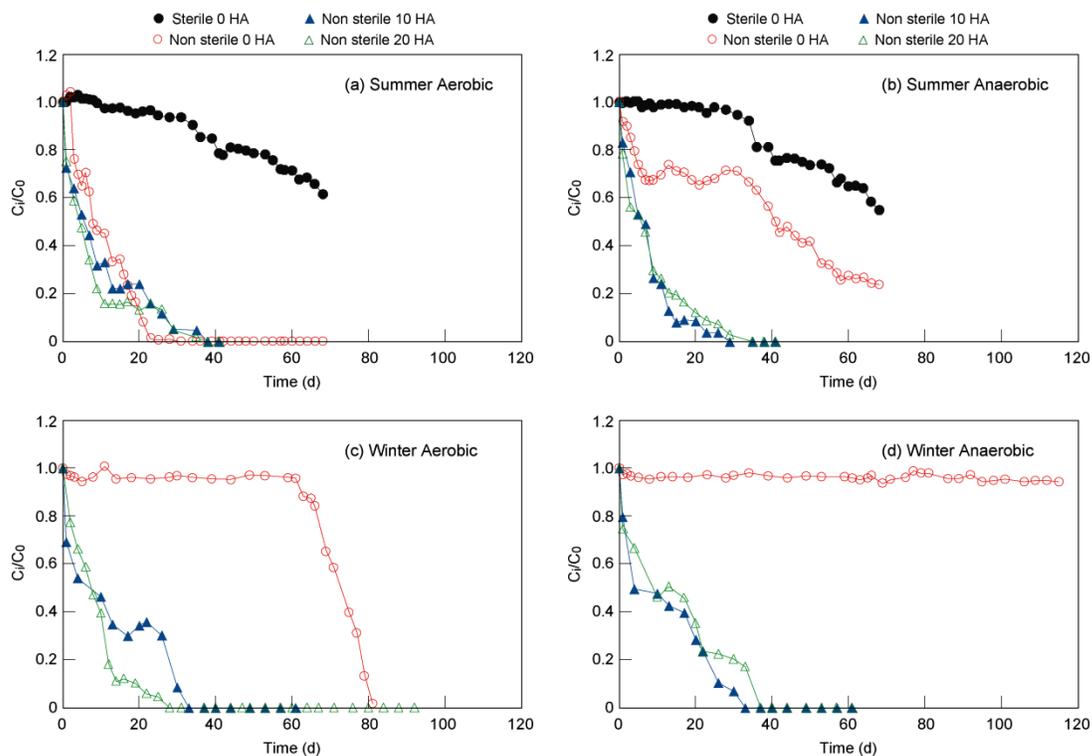


Figure 4. Break through curves of E2 during (a) summer and aerobic, (b) summer and anaerobic, (c) winter and aerobic, and (d) winter and anaerobic conditions.

Similar to caffeine, the removal of E2 was primarily impacted by the presence or absence of HA in the feed water (Fig. 4). In the presence of lake water alone (no HA added) complete removal of E2 was achieved only under aerobic conditions (Figs. 4a, c). In the presence of HA, however, complete removal occurred regardless of the different environmental conditions (Fig. 4). The results suggested that in the absence of HA, removal of E2 was mostly related to biodegradation. Under conditions less favorable for biodegradation, winter and anaerobic, no removal of E2 was observed. In the presence of HA, E2 was removed regardless of the different environmental conditions, suggesting that sorption by HA played a crucial role in the removal of E2. The decrease of E2 in the sterile control observed after 40 days, was due to a loss of sterility.

E1 was impacted by the different environmental conditions in the following order: level of oxygen > level of HA > temperature (Fig. 5). E1 was completely removed only under aerobic conditions. In particular, during the summer E1 was removed regardless of the presence or absence of HA. During the winter, E1 was completely removed only in the presence of a low level of HA.

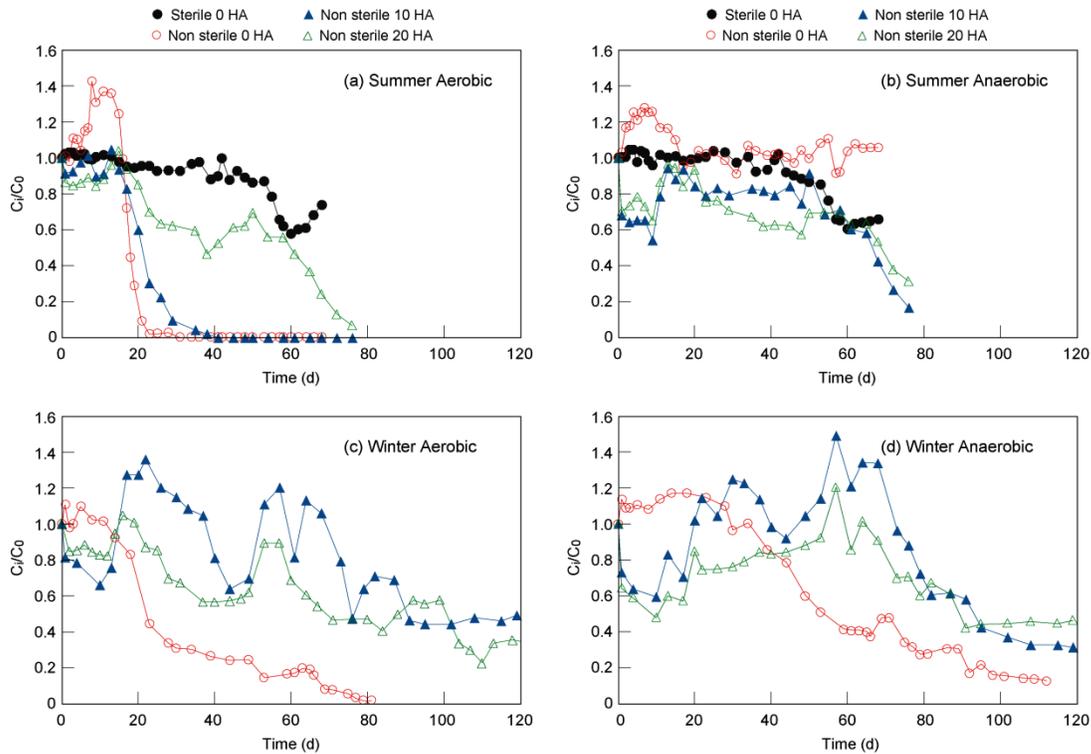


Figure 5. Break through curves of E1 during (a) summer and aerobic, (b) summer and anaerobic, (c) winter and aerobic, and (d) winter and anaerobic conditions.

### Dynamics of Oxygen and Redox Potential in the Sandbox

The dynamics of oxygen in both sides of the sandbox are shown in Figures 6a–b. During the acclimation period, in the presence of filtered lake water alone, three separate zones occurred within each side of the sandbox. In LW, oxygen ranged between 7 and 9 mg/L 10 cm below the water-sand interface (first oxygen probe), between 2 and 5.2 mg/L 50 cm below the water-sand interface (second oxygen probe) and between 2 and 4.2 mg/L 105 cm below the water-sand interface (third oxygen probe) (Fig. 6a). Oxygen was relatively stable during the first 7–8 days, after which it began to drop throughout LW (Fig. 6a). Oxygen decreased from 7 to 3.7 mg/L at the first oxygen probe and from 3.7 to 2 mg/L at the second oxygen probe (Fig. 6a). A constant level of oxygen, 1.8 mg/L, was observed in the lower portion of LW (Fig. 6a).

During most of the first spike, a relatively low and stable level of oxygen, ranging between 2 mg/L near the water-sand interface and 1.8 mg/L at the bottom, occurred throughout LW. A similar behavior was also observed in the second side of the sandbox, LW-HA, fed with lake water and HA (TOC = 20 mg/L). The starting levels of oxygen, however, were slightly lower in LW-HA than LW. Starting levels of oxygen of 7.2, 3, and 2 mg/L were observed at the first, second and third oxygen probe, respectively (Fig. 6a). During the acclimation period, a rapid depletion of oxygen occurred in the top 10 cm below the water-sand interface. Oxygen dropped

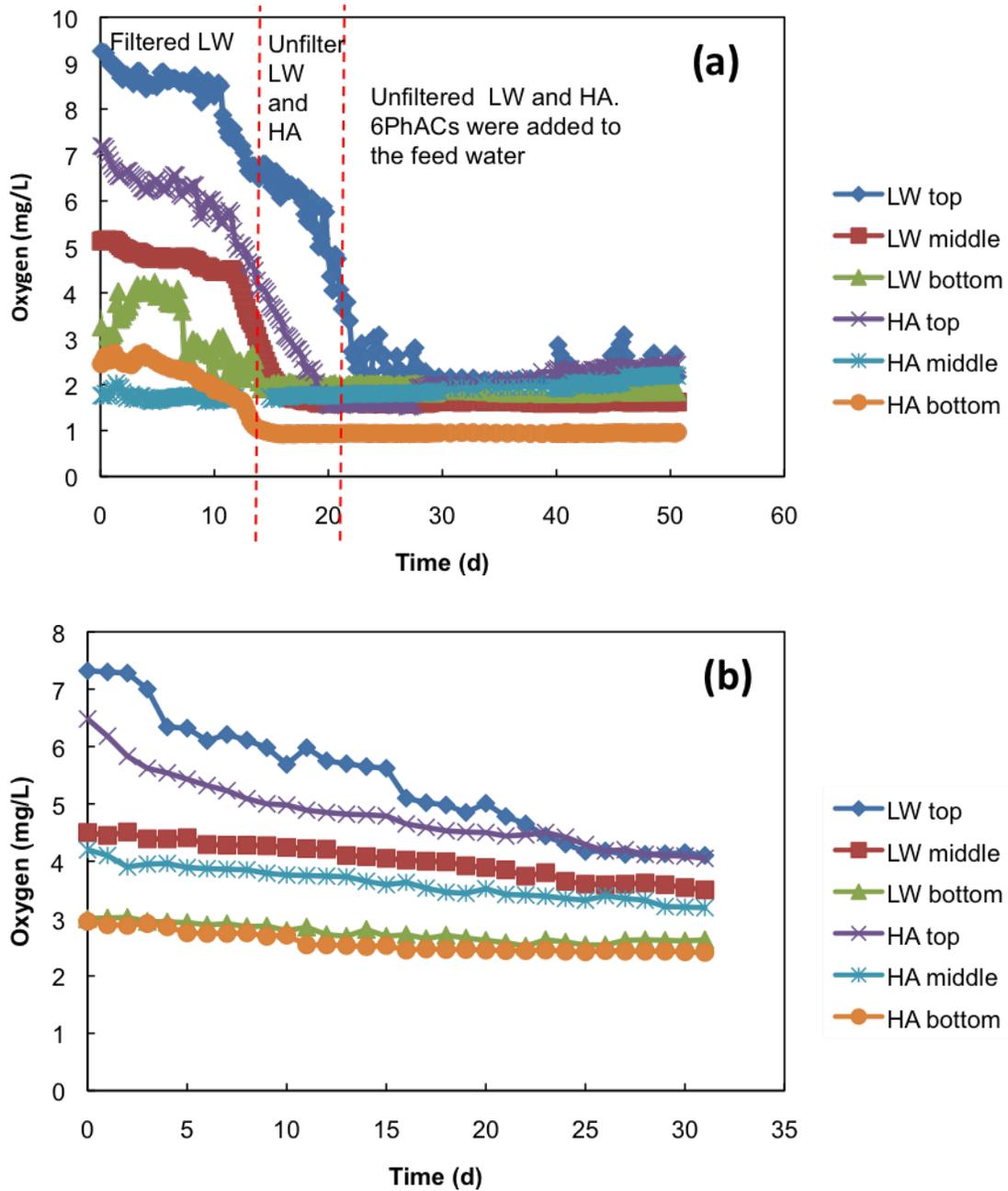


Figure 6. Dynamics of oxygen during the (a) first spike, and (b) second spike of selected PhACs.

from 7.2 to 3.7 mg/L (Fig. 6a). Further depletion occurred during the 5 days (day 17 to day 21) when LW-HA was fed with lake water and HA. Prior to the start of the first spike, on day 21, the level of oxygen was below 2 mg/L at the first and second oxygen probe (Fig. 6a). An even lower level of oxygen (1 mg/L) was observed in the lower portion of LW-HA (third oxygen probe). Oxygen was stable during the spike, ranging from 1.8 mg/L (first two probes) to 0.9 mg/L (third probe) (Fig. 6a).

Prior to starting the second spike, pipettes were used to introduce air into the top section (2–3 cm) of both sides of the sandbox. In LW, starting values of oxygen of 8.2, 4.5, and 3 mg/L were observed after the first, second, and third oxygen probe, respectively (Fig. 6b). During the spike, oxygen was depleted primarily in the upper portion of LW, while a limited reduction occurred at the second and third probe. In LW, at the first oxygen probe which was 10 cm below the water-sand interface, the level of oxygen decreased to 5.8, 4.8, and 4 mg/L after 10, 20, and 30 days of the spike, respectively (Fig. 6b). During the same time, oxygen ranged between 4.5 and 3.7 mg/L at the second oxygen probe and between 3 and 2.5 mg/L at the third oxygen probe of LW (Fig. 6b). A similar trend was observed in LW-HA. The starting concentrations of oxygen were 6.5, 4.2 and 3 mg/L at the first, second, and third oxygen probe, respectively (Fig. 6b). Ten cm below the sand-water interface, oxygen decreased to 5, 4.5 and 4.1 mg/L after 10, 20, and 30 days of the spike (Fig. 6b). No significant depletion of oxygen was observed at the second and third probe. Oxygen ranged between 4.2 and 3.2 mg/L at the second probe and from 3 to 2.5 mg/L in the lower portion of LW-HA (Fig. 6b). Results obtained using the oxygen probes suggested that reduced oxygen conditions existed during the first spike. The introduction of external air before the second spike increased the level of oxygen at least in the upper portion of the sandbox immediately below the water-sand interface.

Results obtained using the sandbox confirmed the results obtained using the columns. Carbamazepine and gemfibrozil were persistent throughout the study, while phenazone was highly impacted by presence of oxygen as well as by the level of HA contained in the feed water. No removal of phenazone occurred in the presence of limited amount of oxygen and in the presence of high level of HA (TOC ~20 mg/L). Partial removal (~50%) of phenazone occurred in the presence of higher levels of oxygen. Removal of caffeine was impacted by the level of oxygen as well as by the presence of HA. Biodegradation was probably the predominant removal mechanism involved. Under aerobic and in the presence of lake water alone, favorable conditions for biodegradation, high removal of caffeine occurred in the top 10–20 cm. In LW-HA, however, presence of HA may inhibit the biodegradation of caffeine. Limited removal of E1 occurred throughout the study, while higher removal of E2 was observed.

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## **Publications in Preparation**

Fate and transport of selected pharmaceutically active compounds during simulated riverbank filtration: Impact of redox conditions, temperature and level of organics. (in preparation)

Impact of infiltrated air on the dynamic of redox conditions as well as on the removal of pharmaceutically active compounds in simulated riverbank filtration during clogging conditions. (in preparation)

# Acquire Sedimentation Data to Promote Reservoir Sustainability and Advance Watershed Science

## Basic Information

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

## Publications

1. Falinski, K.A., D. Penn, An inventory of sedimentation in Hawaii's reservoirs, Journal of the American Water Resources Association. (in preparation).
2. Falinski, K.A., planned June 2016, "Sediment as a Critical Link for Phosphorus Delivery into Tropical Coastal Environments (tentative title)," Ph.D. Dissertation, Department of Tropical Plant and Soil Sciences, University of Hawaii at Manoa. (in preparation)

FINAL REPORT

**Acquire Sedimentation Data to Promote Reservoir  
Sustainability and Advance Watershed Science**

**May 2014**

Dave Penn  
Kim Falinski

Project Number: 2012HI370B

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

## **Abstract**

Hawaii's reservoirs face growing scrutiny due to heightened dam safety and flood control concerns, increasing water demands, and uncertain water pollution effects. In order to promote long term reservoir sustainability, it is vital that we improve our understanding of reservoir capacity loss due to sedimentation. We collected, organized, and analyzed existing physical data about reservoirs located on the main Hawaiian islands. We interviewed reservoir managers throughout the state on the importance of reservoir sedimentation for their reservoir's capacity, and if they were interested in future survey work to understand the rate of sedimentation. Results showed that although sedimentation rate is not documented, it is a serious concern and challenge for reservoir management. Five different management types were identified—federal, state, county, private agriculture and private development. Management practices and reservoir maintenance differed significantly between these groups. Sedimentation, in some cases, was significant enough to lead to dry reservoir conditions. However, unlike in other parts of the world where reservoir sedimentation can be directly correlated with watershed erosion practices, in Hawaii, most reservoirs are partial diversions from streams or from irrigation water systems that are far separated from the watersheds above the reservoir. While engineering surveys consider the structural features of the dam, few also include information about capacity loss. We recommend that dual-frequency bathymetric surveying equipment would provide accurate assessments of sediment depths. Reservoirs in Hawaii are a significant resource for the State's water supply, and maintenance, including assessment and removal of sediments, might be an important consideration for future discussion.

## **Problem and Research Objectives**

Worldwide, one of the leading causes of reduced capacity in small reservoirs is sedimentation (Wang and Hu 2009). Global water use is rising with population and development, while long-term water availability is declining, posing challenges for resource managers. Water needs and constraints in Hawaii follow this pattern (Bassiouni and Oki 2013). With a rapidly growing population, increasing development, and forecast establishment of a diversified agricultural sector for Hawaii, resource managers and planners must carefully consider how to meet growing demand while balancing human welfare, economic impact, and ecological sustainability (Water Resources Associates 2003). Reduced capacity has real economic costs, including less available water, dredging costs, greater risk of breach, and lower capacity for electricity generation (Hawaii Department of Agriculture 2010).

On the other hand, small reservoirs have also been shown to be effective sediment retention basins, and may positively impact downstream water clarity and quality. Small reservoirs have been shown to increase water availability, reduce peak flow storm runoff and improve water quality (Deitch, Merenlender et al. 2013), and small dams can be beneficial to aquatic ecosystems, because they can reduce the amount of sediment and nutrients carried in surface runoff (Verstraeten and Prosser 2008). Sediment can result from natural processes and human disturbance; sediment loads are directly related to land use, land cover, geology, and climate. The ability of the ecosystem to retain sediment is a key ecosystem service.

Reservoir sedimentation data has been used in other places to better understand which watershed characteristics contribute to sediment and nutrient export. While sediment yield in watersheds is connected to some physical parameters, including area, soil type and rainfall characteristics, land use and management have been shown to contribute to sedimentation processes as well. For example, similar work by Verstraeten et al. (2003) used reservoir sediment accumulation data to validate the advantages of assessing catchment sediment yield through qualitative and quantitative approaches that incorporate more complex variables than just catchment size and generalized soil erosion rates. In Hawaii, it remains unclear which watershed physical characteristics, and land use and management actions are variables that contribute to overall sediment export (Hoover and Mackenzie 2009; Storlazzi, Field et al. 2009). Additional sediment export data is needed to calibrate and validate sediment export models to be able to draw watershed-scale conclusions, and reservoirs provide data that integrated over longer periods of time than most in-situ sampling efforts can achieve.

Regardless of whether reservoir sedimentation data are used to describe a decrease in water storage capacity or to better understand watershed processes that contribute to sediment export, an understanding of reservoir sedimentation processes is critical to surface water management. The United States Geological Survey (USGS) collects nation-wide information about reservoir sedimentation for the Reservoir Sedimentation Database (RESSED). The database does not currently contain any records for Hawaii reservoirs.

The objectives of this project:

- 1) Obtain information pertaining to Hawaii reservoir sedimentation, including design and as-built bed elevations, drainage area sediment yield, and source sediment,
- 2) Obtain corresponding data from subsequent watershed analyses and hydrologic calculations,
- 3) Organize the collected information into a database that mimics the structure of the national reservoir sedimentation database (RESSED),
- 4) Perform a gap analysis of the database records to identify remaining data needs,
- 5) Develop a sampling and analysis plan for a field and archival investigation to the fill gaps in the historic record and to establish new baselines for reservoir physical characteristics, and
- 6) Assess the vulnerability of Hawaii's fresh and coastal water resources.

## **Methodology**

### **Data Acquisition**

We obtained permission from the U.S. Army Corps of Engineers to access the National Inventory of Dams (NID) via its secure portal. At an initial meeting with the staff of the State of Hawaii Dam Safety Program (HDSP), we obtained similar permission to access the HDSP inventory. The NID and HDSP have information about the size, capacity, spillway characteristics, year built, and ownership of most of the 140 reservoirs in Hawaii.

In addition, we conducted literature searches and gathered references that document reservoir-specific physical characteristics (e.g., geotechnical investigations, dam safety inspections, emergency action plans, and environmental assessments). We tracked legislative and regulatory

initiatives and agency reporting of dam and reservoir activities, including budget appropriations, revenue proposals, compliance and enforcement actions, and dam removal permit status. Specifically, we collected all of the reports available publicly to the legislature from 2007 to 2013 describing changes in the dam status. Every attempt was made to assemble private reports on each reservoir, as permissible by the consulting companies that wrote the reports.

## Database Creation and Analysis

We first created a database in Microsoft Access that mimics the structure of the national RESSED database for data input. We then combined and cross-checked the data from both the NID and HDSP databases with the RESSED fields. There were some fields that were added that are not in the RESSED structure, but allow for more detailed analysis of the dataset. A screenshot of one of the databases entry forms is presented in Figure 1.

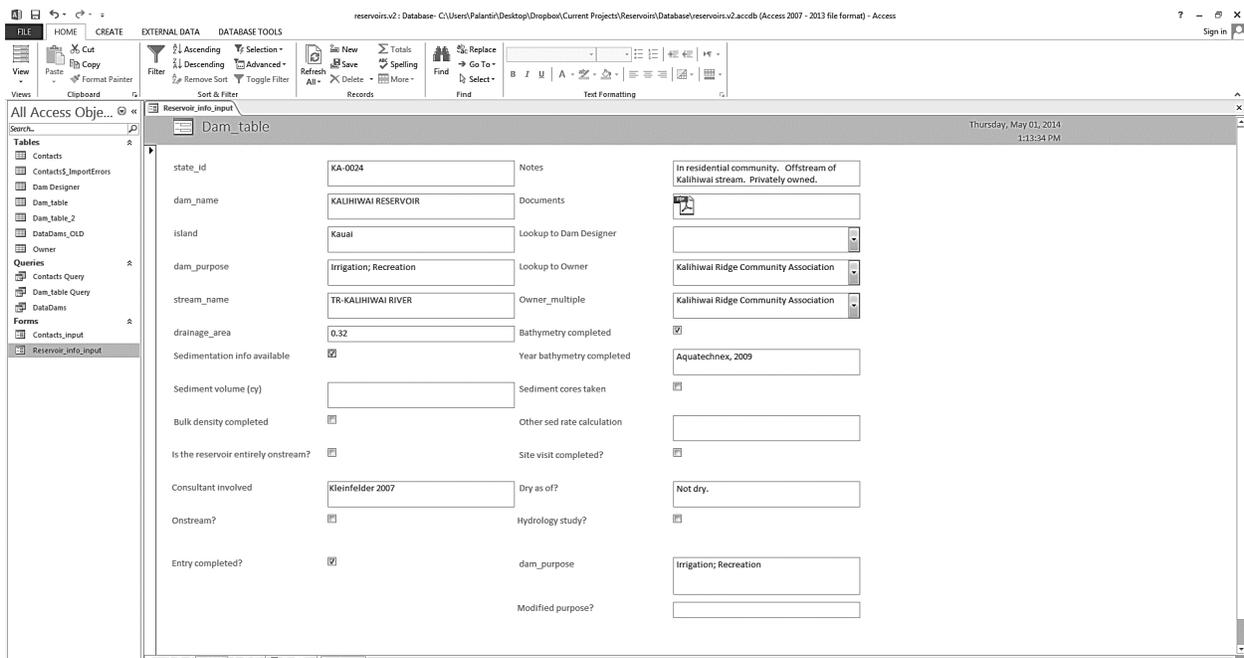


Figure 1. Microsoft Access database reservoir entry form.

After assessing the types of information that are available in the NID and HDSP inventory records we sorted the inventory database records by location, owner, size, and use type. Because of the nature of this assessment, we conducted an analysis to better describe the reservoirs of Hawaii so that we could make generalizations about the susceptibility to sedimentation problems and to create a sampling plan that would be practical. The results of that analysis are presented here.

## Interviews with Reservoir Owners

Using the HDSP inventory, we contacted the 42 dam owners and operators, covering the 140 reservoirs in the HDSP inventory and to participate in our project by mail. The introductory letters were followed by phone interviews. The survey used in the interviews was conducted for each reservoir that the owner/manager was responsible for. (see Table 1). Interviewees are not identified by name in this report.

Table 1. Survey instrument used with Hawaii’s regulated reservoir owners and managers.

	Question	Answer format
1	When was the last time your reservoir(s) was (have been) surveyed/measured?	Year
2	Original plans available?	Yes/no
3	As-built drawings available?	Yes/no
4	How many times has it been surveyed?	Number
4a	Who did the survey?	Name
4b	What method?	Acoustic, bathymetric survey, sediment sampling,
4c	How much did it cost?	Number
5	Why did you do it?	Open
6	What will you do with the results?	Open
7	Are you willing to share those results with us?	Yes/no
8	What is your level of interest in conducting new surveys/measurements in order to find out how much the reservoir has filled in with sediment? (1–5)	Scale (1–5)
9	Would you like to continue to talk with us and help us gather more information on reservoir sedimentation in Hawaii?	Yes/No

We conducted site visits to publicly and privately owned reservoirs on the islands of Oahu, Maui, and Hawaii, and gathered other information through email and phone calls. The site visits were primarily to assess and photograph the extent of sedimentation through visual surveys and to gather hard copies of documents describing sedimentation. There was significant collaboration with the Hawaii Department of Agriculture’s (HDOA) Agriculture Development Corporation (ADC) to obtain information about reservoirs, including visits to archival libraries of information.

## Principal Findings and Significance

There were 140 regulated reservoirs in the state of Hawaii at the time of this study, yet only one reservoir (out of 140) in the state had enough information to add the fields required by the national RESSED database: Kaneohe reservoir on Oahu (Wong 2001). Additional studies, however, have been conducted on 34 reservoirs by private engineering firms to maintain compliance with the Department of Land and Natural Resources (DLNR) dam safety program. We discovered that each reservoir has a unique history of maintenance, sediment build-up and water use, and that to acquire sedimentation data for any of the reservoirs requires a detailed, recorded history of how it was managed. The types of documents available varied widely, but formal sedimentation surveys are not typically conducted as part of engineering studies. Evidence is mostly anecdotal.

Our interviews indicate that most reservoir managers have experienced some level of sedimentation issue with their reservoir, and that others have developed methods to mitigate the effects of sediment build-up. Since the 2007 breach of Kaloko dam, many owners are shifting towards decommissioning their reservoirs, but sediment build-up is not cited as the driver for the changes.

## Data Analysis of Reservoirs in Hawaii

The majority of reservoirs in Hawaii are used for irrigation purposes (80%), as seen in Figure 2. Categorical analysis made some calculations difficult, especially when reservoirs were listed with multiple uses (this occurred in 26 out of 140 dams). An example is Alexander reservoir in Kauai, which is used for both hydroelectric power generation and for irrigation. Multiple uses of reservoir water allow for multiple benefits to the hydrologic systems.

Compared to other worldwide locations where significant information for watershed sedimentation processes are collected from sediment deposition rates, 80% of the reservoirs used for irrigation in Hawaii often have water delivered far from the reservoir, or only a portion of the stream is used to supply the reservoir. These off-stream, and sometimes off-ditch reservoirs do not offer enough information to be able to discern larger watershed processes, but are still relevant to understanding the loss of capacity due to sedimentation.

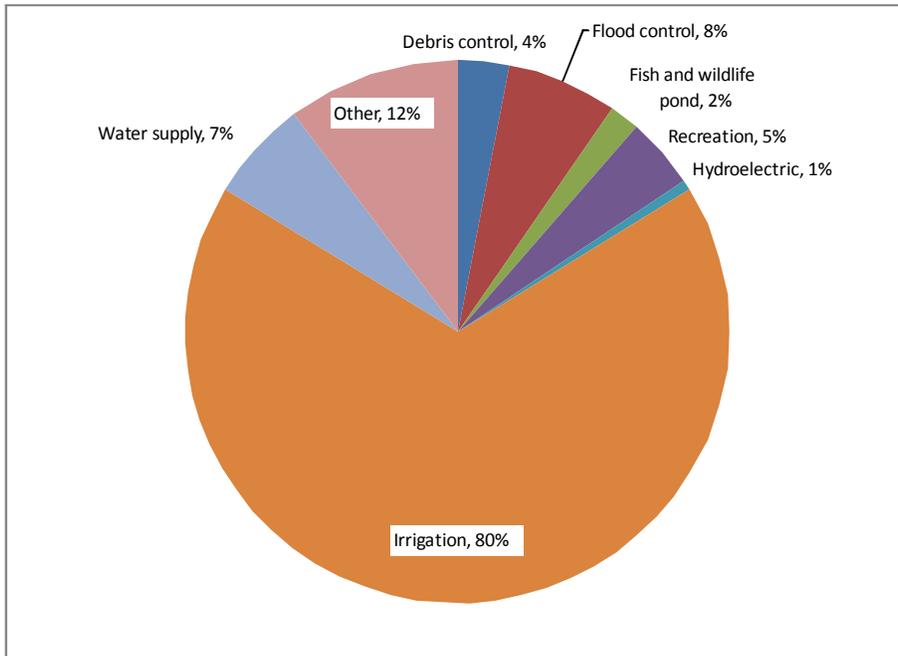


Figure 2. Percentage of reservoirs in Hawaii by use type.

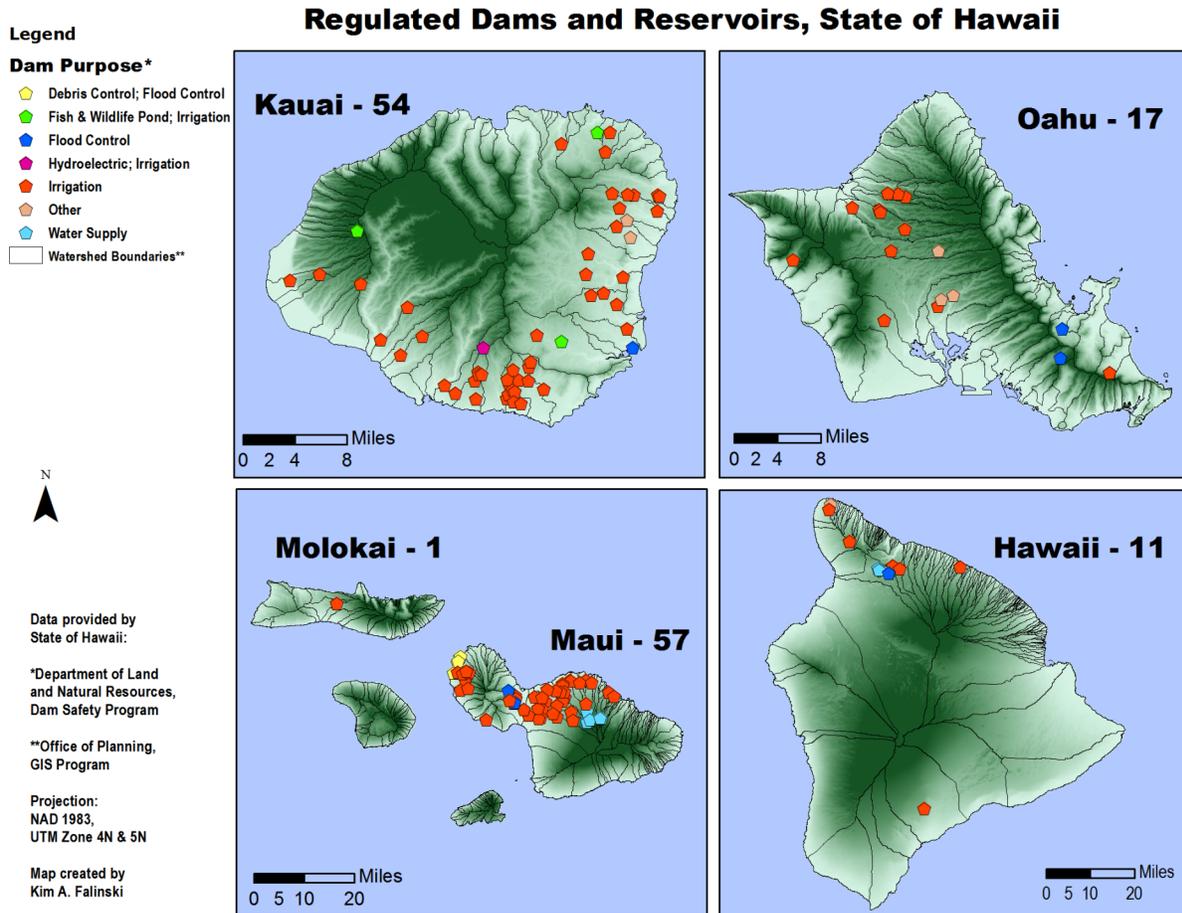


Figure 3. Reservoirs classified by use type for Kauai, Oahu, Molokai, Maui, and Hawaii. Note: The total number of reservoirs found on each island (as of March 2013) is listed next to the island name.

Data describing the current water storage and the maximum storage by island, is presented in Table 2. This information is important when considering the risk of sedimentation to Hawaii’s reservoirs. Only the Molokai reservoir is used for both irrigation and water supply and is kept close capacity. In general, it was found that the reservoirs were only kept close to capacity when used for water supply (80%) or for fish and wildlife ponds (97%). Irrigation reservoirs, comprising 80% of the used storage, were kept overall at 67% capacity. The one hydroelectric dam currently regulated in Hawaii is operated at 42% capacity, but conversations with the owner indicate that sediments are a problem and are contributing to the reduced storage. These figures are important, and may be of critical concern, as they indicate that storage loss is due to sedimentation. Although 64% of the reservoirs are considered small in size, 74% of the capacity is provided by intermediate sized reservoirs (n = 49).

Table 2. Normal and maximum reservoir storage by island.

	Normal Storage (ac-ft)	Max Storage (ac-ft)	Percent of Max
Hawaii	1242.4	2984	42
Kauai	15553.1	27460.1	57
Maui	4346.97	6768.86	64
Molokai	4265	5082	84
Oahu	10803	20799	52
Total	36210.47	63093.96	57

Table 3. Data for reservoirs use type.

Reservoir Type	Normal Storage (ac-ft)	Max Storage	Number of Reservoirs	Percent of Total	Percent Capacity	High or Proposed High (%)
<b>Debris control</b>	155	626.4	5	4	25	100
<b>Flood control</b>	1052.37	11180.77	11	8	9	91
<b>Fish and wildlife pond</b>	1436	1480	3	2	97	67
<b>Recreation</b>	1991	9878	7	5	20	100
<b>Hydroelectric</b>	1070	2540	1	1	42	100
<b>Irrigation</b>	31583.3	47153.99	112	80	67	88
<b>Water supply</b>	5104.8	6385.2	10	7	80	80
<b>Other</b>	3395	4583	17	12	74	82

**Owner and sediment:** The majority (by number) (70%) are reservoirs are owned in part by private owners, including large farms (56%) and a diverse array of owners, including small farms, housing developments and recreation and tourism facilities (14%). The owner types presented in Figure 4 were established based on careful consideration of the data. Private-Other owners are private owners who manage three or fewer reservoirs. This group is more likely to be using the reservoirs for development or other urban reservoir uses, including for aesthetic reasons Unlike Private-Agriculture, smaller private owners are less likely to have the resources to repair or excavate reservoirs.

**Drainage area:** The quality of the data in the drainage area field in the HDSP database is variable. The irrigation systems in Hawaii are complex and not always well documented. During

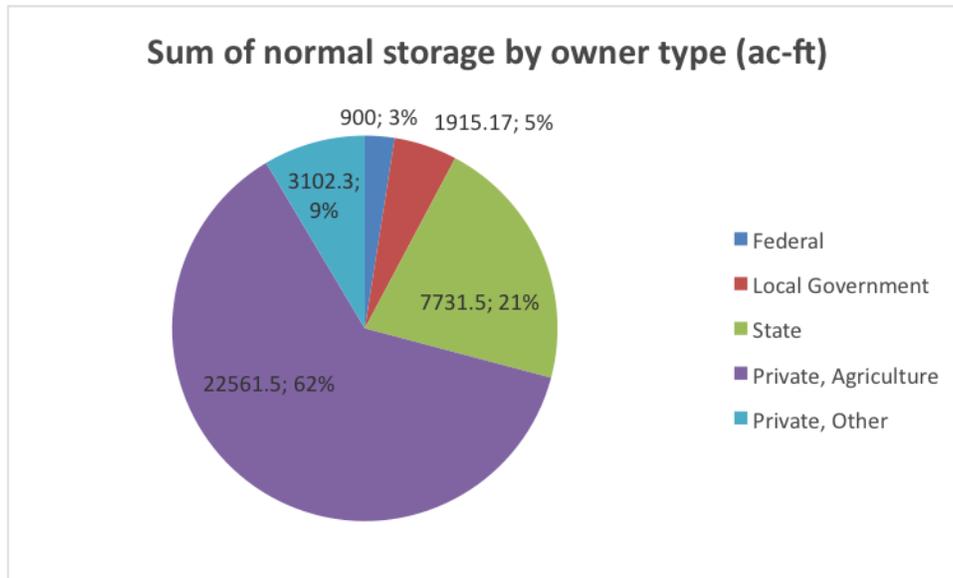


Figure 4. Storage capacity of Hawaii’s reservoir by owner type.

this investigation, we found many reservoirs that were spatially disconnected from the watersheds that provided the water.

**Safety status:** The current database shows that 88% (n=123) of all of the dams are considered to have a high or proposed high safety status according to DLNR. Only 2% of the reservoirs were deemed to have a significant risk factor (n=3), and 6% (n = 9) were considered to be a low risk factor.

**Decommissioning patterns:** The overall number of reservoirs in the state of Hawaii is in a steady decline. Irrigation reservoirs, which make up a majority of the reservoirs, have been decommissioned in the last five years at a disproportionately high rate, yet it was determined that sedimentation is not the highest concern of reservoir managers.

The decommissioning of reservoirs that are located within the critical irrigation systems and are of particular concern are: the Pioneer Mill irrigation system in west Maui, the Waimea irrigation system on the Big Island, and the East Kauai irrigation system in Kauai. Interviews and field visits suggest that sedimentation is not the primary issue for compliance with safety regulations, but dams are vulnerable to aging infrastructure. Historically, these reservoirs were maintained through public-private partnerships, but lack of finances and demand combined with a more robust dam safety regulatory structure led to their disuse. This is a disservice to the water capacity of the islands as a whole, in part because the reservoirs.

**Unregulated dams:** Through our consultations with HDSP and the regulated reservoir community, we discovered that there are at least 300–400 unregulated dams with no reporting requirements that would otherwise provide information to facilitate the achievement of watershed research objectives. The reservoirs, ponds, and basins associated with these

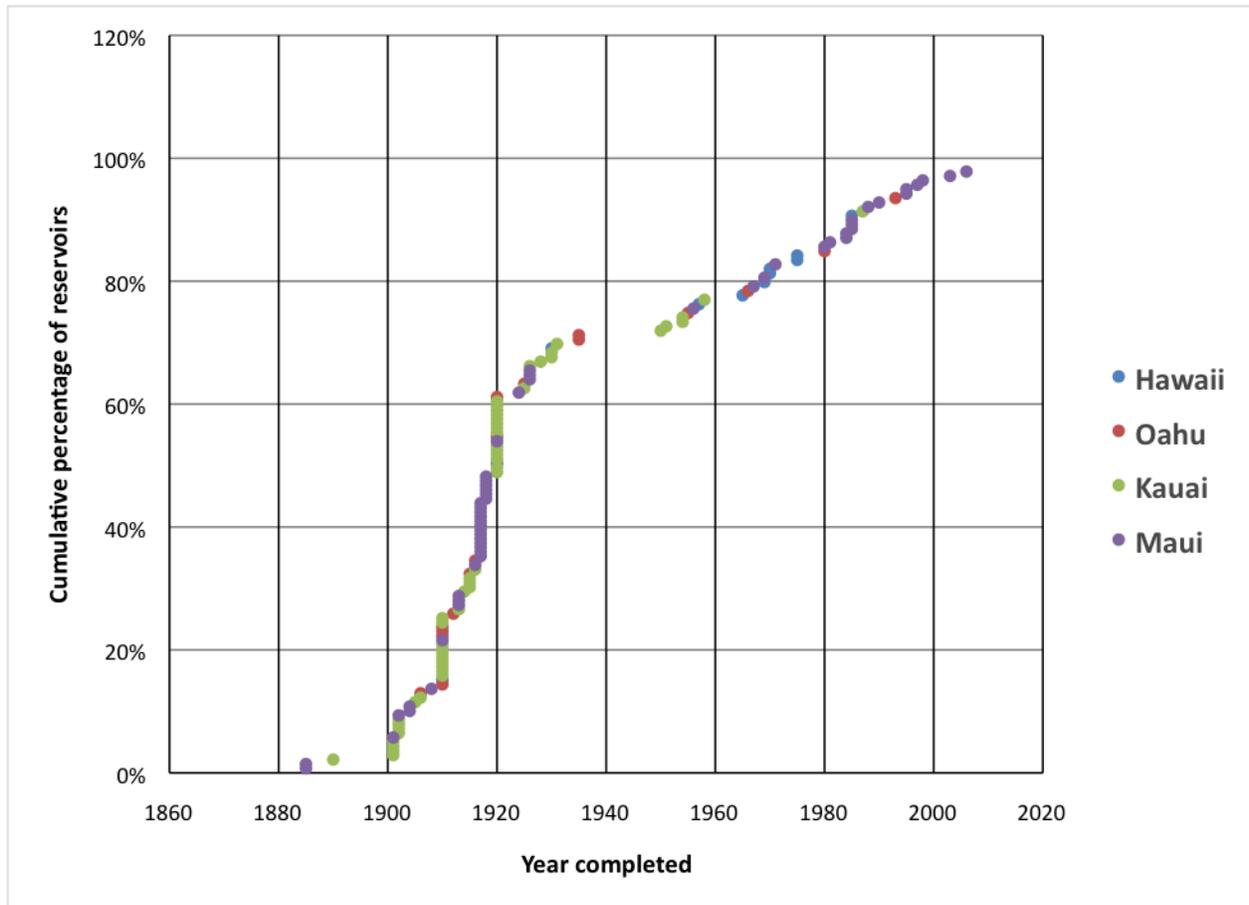


Figure 5. The age of reservoirs, by county of Hawaii. Note: Nearly 60% of the reservoirs were built by 1920. The most recent dams have been constructed for flood prevention and sediment retention.

unregulated structures could actually provide a more manageable and robust nexus for watershed science research than would the larger, more complex systems associated with regulated dams (Verstraeten and Poesen 2001; Verstraeten and Poesen 2002)

**Age of reservoirs:** Figure 5 presents the cumulative age of reservoirs for the four counties of Hawaii. Many of Hawaii’s reservoirs are approaching 100 years old. Interviews suggested that while the sugarcane plantations frequently dredged out small reservoirs so that they could hold their full capacity, large reservoirs were likely never dredged unless they prevented the intake or outflow pipes from operating successfully (Kauai Ranch, personal communication). No new reservoirs have been built since 2006, and a majority of the reservoirs built in the last three decades were built on Maui.

**External evaluations:** Several consulting engineering companies are involved in assessing reservoirs. In some cases, these companies are primarily performing structural analyses and presenting recommendations for maintaining the reservoir in a safe condition. However, few engineering analyses included a survey of sedimentation, or made adjustments for capacity loss due to sedimentation. This is especially a concern for private owners that are not agricultural.

Private agricultural companies have the heavy earth moving and forming equipment available, and can also divert water to allow the reservoir to be dredged. The dredge spoils can be then placed on land. Smaller private developments, though, do not have these resources.

**Availability of information:** Public works reservoirs and those owned by large corporate owners had the most complete information sets.

### Interviews with Reservoir Owners

Table 4 describes the number of respondents by owner type, and their responses to question 8: *What is your level of interest in conducting new surveys/measurements in order to find out how much the reservoir has filled in with sediment? (1-5)*

Reservoirs that are owned by the local county or state-owned, had the most documented information available; 7 reservoirs that were local or state owned had partial information that will be provided to the RESSED database. (e.g., The Hawaii Department of Agriculture and Board of Water Supply both have detailed information about their respective reservoirs).

Notably, many former or current large agricultural land owners with multiple reservoirs would have a single point person who manages the reservoir. These owners did not have many written records available, but had built-up expertise and knowledge within the organization over time. Larger agriculture owners acknowledged sedimentation, actively used reservoirs as detention basins, and had protocols and schedules to dredge sediments. There is a lot of potential with this group to obtain more formal records and to do on-site surveys of sedimentation rates.

On the other hand, individuals, trusts or partnerships that generally managed only one or two reservoirs (private/other) are struggling to understand the required regulations, and have invested significantly in private engineering reports to substantiate the safety of their dams.

Table 4. Summary of survey responses by owner type.

	Federal	State	Local Government	Private-Agriculture	Private-Other	Total
Number of owners	1	4	6	9	15	35
Number responded to survey	1	3	2	4	10	20
Mean interest level	5	4	5	5	3.4	

Many of the interviewees noted that there is currently no ideal way to remove sediment from reservoirs. As it stands now, reservoir managers need to drain the reservoir, which may take several weeks, and then manually remove sediment using backhoes and other mechanical equipment. If the reservoir was not originally designed to allow access for these types of vehicles, then the sediment must be removed manually. This was done recently at Waiakoloa reservoir on the Big Island. If the landowner does not have a use for the excavated sediment, there is also the issue of disposal. Recent controversies surrounding using dredged sediments for fill and restoration of agricultural land limit the ability for owners to deposit the sediments elsewhere. Issues of sediment-sorbed contaminants may prevent movement of the sediments to other properties or landfills.

## Case Studies

Wahikuli reservoir and Kitano reservoirs were reported as heavily silted in the 2003 Agricultural Water Use report (Water Resources Associates 2003). Wahikuli reservoir has since been breached and decommissioned, and it was estimated to cost \$2.19 million to dredge and rehabilitate.

Reservoir 155 on Oahu, which is the end point for the Waiahole Ditch system, was reported to have significant sediment build-up. There was an estimated projected cost of \$566,000 for the removal of sediments and repairs for sediment-related damages.

In most cases, sedimentation is not the only limitation to water capacity. Most of the aging reservoirs require the installation of new-HDPE style liners (or equivalent) to prevent seepage, in addition to making structural improvements to earthen embankments that may have degraded over time.

Lower Hamakua ditch was reported to have an approximate one-foot depth of sediment build-up, when it was refurbished in 2004, indicating that while ditch systems are unlikely to represent natural erosion processes, channel erosion from the walls of decaying ditch systems does allow for sediments to enter and build up in the receiving reservoirs. For the Waimanalo ditch system on Oahu, interviews revealed that hired workers manually clean the ditches of silt and debris on a daily basis.

## Discussion

### Data Incomplete on Sedimentation and Bed Elevation

The level of information available for bed elevation and other reservoir characteristics varies widely; it would take considerable effort (beyond the resources of the current project) to track down reasonably complete information about each reservoir. It was determined that bed elevation is not routinely included in the inventories, databases, and documents. In some cases, it may be derived from existing inventory data about dam height, site elevation, and reservoir depth (which by itself must generally be derived from reservoir surface area and storage

capacity). However, initial estimates of original bed elevation are available for reservoirs that were the subject of previous 1970s-era national program investigations, which include area-capacity curves that indicate a base elevation at which storage capacity is zero (Harding-Lawson Associates and Department of the Army 1978). Regardless of where the bottom of the reservoir is located is a repeated concern amongst owners and operators that we spoke with.

## Actions of Dam Owners

Many owners and operators recently performed, or are currently performing, new investigations to support dam removal and compliance with newly revised dam safety regulations (Hawaii Administrative Rules Chapter 13-190.1) (2001). For example, from July 2010 to June 2012, ten dams were at some stage of the permitting and construction process for removal, and the state did not proceed with any enforcement actions against dam owners. However, these investigations typically do not include measurement of reservoir bathymetry and determination of base elevation and changes in bed elevation over time. Although owners and operators that we talked with are interested in this information, a more driving concern—and one which may hold promise for future research—is to identify and employ effective and efficient sediment removal methods that meet environmental protection requirements. “Draining dams may be good from a safety standpoint, but it’s not good for agriculture.”

## Current Legislative Issues Regarding Reservoirs and Dam Safety

Re-developing agriculture in Hawaii will require reliable access to water for irrigation. Many reservoirs act like sediment retention basins, and prevent sediments from delivery and transport to coastal waters, thereby preventing possible water quality problems. The State legislature explicitly prioritized maintaining agricultural land for local food supply, economic growth and jobs, tourism and aesthetic reasons. Agriculture in many parts of the islands depends on a steady irrigation supply, yet Hawaii’s 140 reservoirs that store water for irrigation are being decommissioned and breached at a rapid rate. This is due to the increased regulations following the Kaloko dam break in 2007 in Kauai, and because of the risk of structural instabilities due to the poor maintenance of the irrigation systems created in the 19<sup>th</sup> century that also included sedimentation and debris clogging to the inlet and outlet structures.

Funding is a critical issue for all aspects of reservoir sustainability. In 2012, Hawaii voters rejected a proposed amendment to the state constitution that would have authorized the state to issue special purpose revenue bonds and use the proceeds from the bond issue to assist dam and reservoir owners to make their facilities compliant with current safety standards). 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 lobbied for again next year

## Future Work

The main objective of our research is to connect information about reservoir sedimentation with analysis of watershed processes—particularly in relation to sediment yield characteristics—and related watershed management issues, such as irrigation supply, flood control, in-stream flow requirements, and aquatic habitat quality.

The State of Hawaii may not have sufficient resources and interest to support the development and implementation of extensive reservoir sustainability measures. It remains to be seen how many more dams will be removed due to safety concerns and economic constraints. However, sediment deposition in reservoirs is an ongoing problem—leaving issues unaddressed until problems become acute leads to costly and/or hasty ineffective solutions (Randle, Collins et al. 2013).

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### **Student Support**

This project supports PhD student Kim Falinski through May 2013. The research from this project will be incorporated into her dissertation.

# Bioaccumulation and Biotransformation of Arsenic by Marine Algae in Hawaii

## Basic Information

<b>Title:</b>	Bioaccumulation and Biotransformation of Arsenic by Marine Algae in Hawaii
<b>Project Number:</b>	2012HI389B
<b>Start Date:</b>	3/1/2012
<b>End Date:</b>	2/28/2014
<b>Funding Source:</b>	104B
<b>Congressional District:</b>	Hawaii 1st
<b>Research Category:</b>	Water Quality
<b>Focus Category:</b>	Water Quality, Surface Water, Toxic Substances
<b>Descriptors:</b>	
<b>Principal Investigators:</b>	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 $\mu$ ) 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  $\mu$ l was used. Yttrium prepared at a concentration of 150 ng/ml in mobile phase was employed as an internal standard. Injection of 10  $\mu$ 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 <sup>+5</sup> ) as Arsenic	Arsenite (As <sup>+3</sup> ) 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 (Ar<sup>40</sup>Cl<sup>37</sup>), 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 Ar<sup>40</sup>Cl<sup>37</sup> 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 amendable 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.

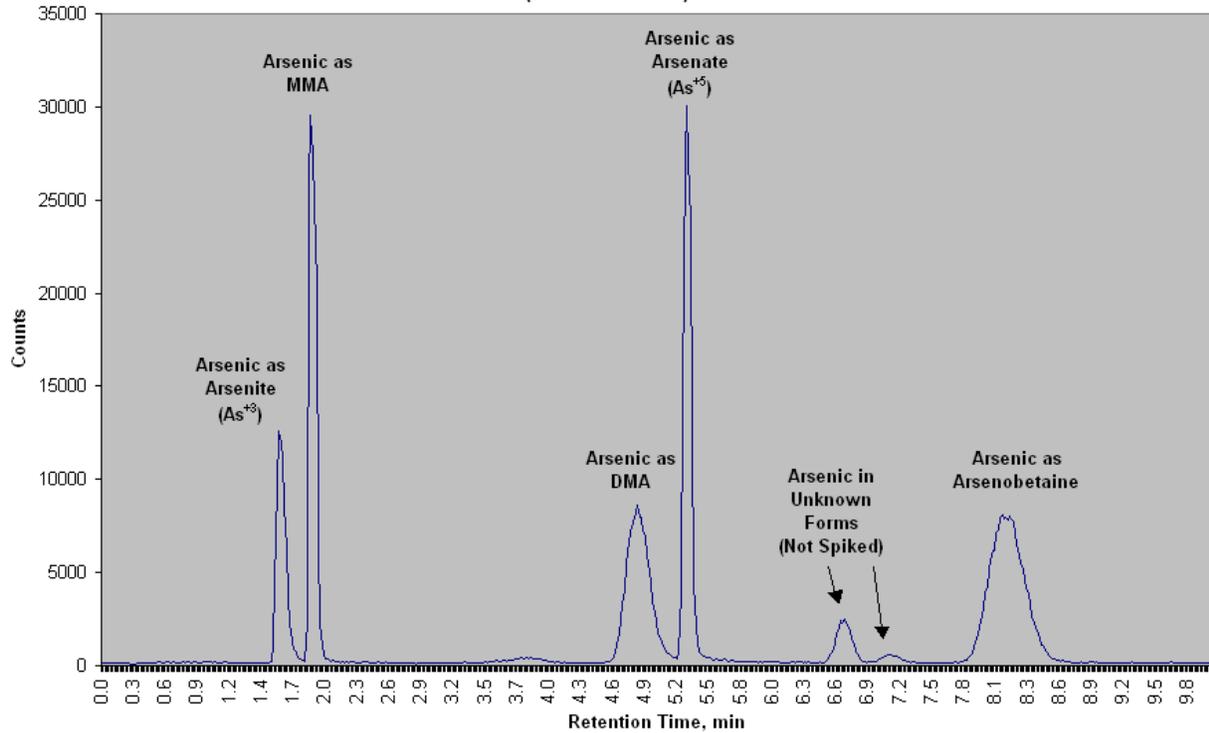
**Total Arsenic Analysis:** Total arsenic analysis was performed with a VG Elemental PQ Excel ICP-MS. Yttrium was utilized as an internal standard and the instrument was calibrated daily with reference materials procured from SPEX Certiprep. Verification of instrument calibration was achieved with preparations 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)

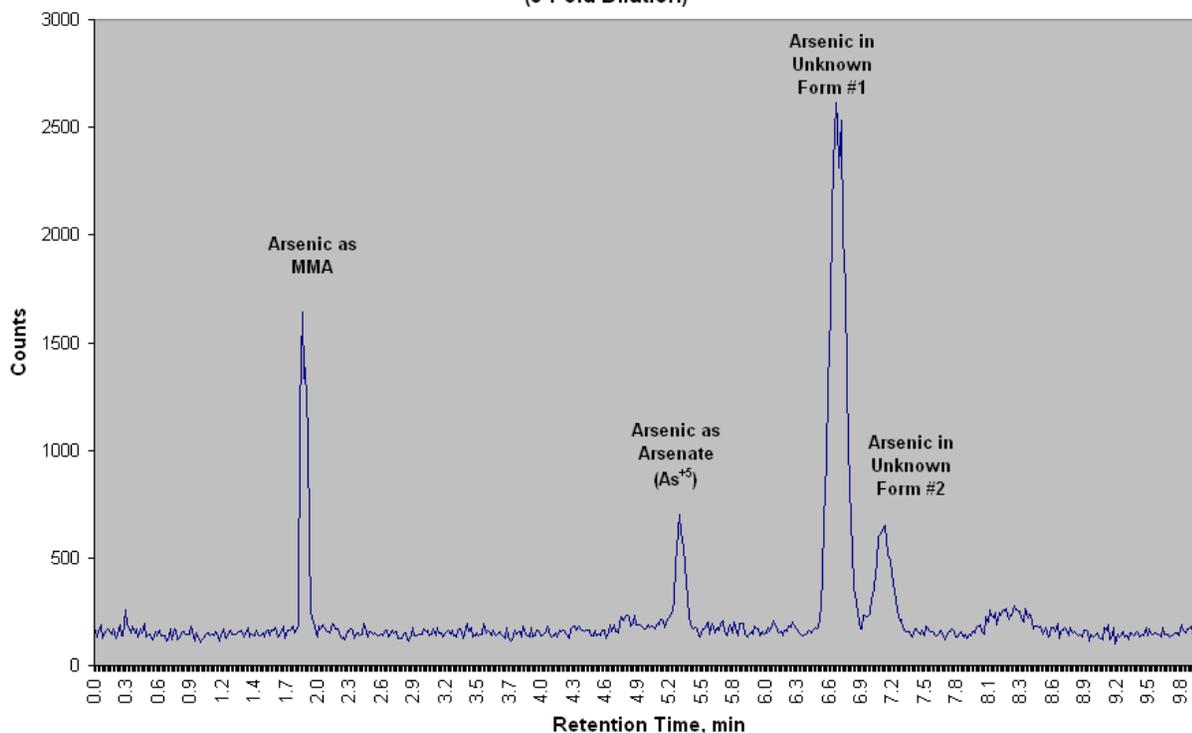


Figure 2 presents the LC-ICPMS chromatogram of an alga extract with no species spike.

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  $\mu\text{g/g}$ .

**Table 1: Final Arsenic Species Results for Algae Extracts**

	Total Arsenic*, $\mu\text{g/g}$	Arsenite, $\mu\text{g/g}$	MMA, $\mu\text{g/g}$	Unknown Species #1, $\mu\text{g/g}$	DMA, $\mu\text{g/g}$	Arsenate, $\mu\text{g/g}$	Unknown Species #2, $\mu\text{g/g}$	Unknown Species #3, $\mu\text{g/g}$	Arsenobetaine, $\mu\text{g/g}$	Species 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).

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## Award--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

<b>Title:</b>	Award--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
<b>Project Number:</b>	2012HI412S
<b>Start Date:</b>	9/1/2012
<b>End Date:</b>	10/31/2013
<b>Funding Source:</b>	Supplemental
<b>Congressional District:</b>	Hawaii 1st
<b>Research Category:</b>	Ground-water Flow and Transport
<b>Focus Category:</b>	Groundwater, Water Supply, Models
<b>Descriptors:</b>	
<b>Principal Investigators:</b>	Aly I El-Kadi

### Publication

1. 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, depend on the long-term sustainability of the groundwater resources in the Pearl Harbor Aquifer.

For proper resource management it is critical 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 Kalauao 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 focuses 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 (Bakker and Schaars 2005).

The model developed for this project incorporates the latest available groundwater-recharge estimates developed by the USGS, recent groundwater withdrawal rates, and aquifer parameters that are based on previously published data. 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 the leeward and Pearl Harbor side in the Koolau high-level water area can also 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 were used to characterize the effectiveness of the alluvium as a hydrologic barrier. Moreover, continuously measured water levels were 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**

The Saltwater-Intrusion model code was successfully used in one-dimensional cross sections to test the suitability to simulate density-dependent groundwater flow in freshwater-lens aquifer systems. Hawaii aquifers are vastly heterogeneous. To develop an island-wide model, Oahu's complex geology has been reduced to three simplified hydrogeologic units: (1) dike-intruded volcanic rock, (2) dike-free volcanic rock, and (3) sediments and rejuvenated Volcanics. The altitude of the contact between overlying sediments and volcanic rock has been digitized for the entire island to 6,000 ft below sea level, which facilitates the model grid generation of the hydrogeologic units. Published water levels from wells, tunnels, springs, and wetlands on Oahu were compiled to calibrate the numerical model. To date, results have not been finalized as availability of the USGS recharge estimates for Oahu has only been made available on May 2014.

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# Evaluation of the Impact of Drought Conditions Upon the Waiahole Ditch System Development Tunnels: Ground Water Sustainability Implications Under Adverse Climate Change Conditions

## Basic Information

<b>Title:</b>	Evaluation of the Impact of Drought Conditions Upon the Waiahole Ditch System Development Tunnels: Ground Water Sustainability Implications Under Adverse Climate Change Conditions
<b>Project Number:</b>	2013HI415B
<b>Start Date:</b>	3/1/2013
<b>End Date:</b>	2/28/2015
<b>Funding Source:</b>	104B
<b>Congressional District:</b>	First
<b>Research Category:</b>	Climate and Hydrologic Processes
<b>Focus Category:</b>	Drought, Climatological Processes, Hydrology
<b>Descriptors:</b>	None
<b>Principal Investigators:</b>	Aly I El-Kadi

## Publication

1. There are no publications.

## **Introduction**

The study concerns understanding the negative effects of climate change on the Waiahole Ditch System, high-level dike compartment tunnels and its associated regional distributive down-gradient recharge that affects the sustainability of the Pearl Harbor basal aquifer. The research area encompasses the Waiahole Ditch System (Fig. 1) and the Pearl Harbor aquifer (Fig. 2). In their evaluation of these dike systems, Takasaki and Mink (1985) provide a historical framework of investigations into this phenomenon, with their estimate at the time, of 560 billion gallons of storage occurring within the primary recharge area of the Koolau Mountains—with no recharge estimate provided at the abutment to Pearl Harbor. Since the Pearl Harbor aquifer is heavily utilized by various entities, with major withdrawals by the Department of Defense and the Honolulu Board of Water Supply (HBWS), recognition of climate change associations may assist policy makers in determining appropriate groundwater adaptive strategies to meet those challenges.

## **Problem and Research Objectives**

Reductions in recharge due to adverse climate change will affect the predominant base flow contributions from the Waiahole Ditch System development tunnels, re-kindling competition for this valuable resource among leeward and windward interests. Adverse climate change influences manifest by Waiahole Ditch discharge reductions will also have its associated impacts upon the groundwater flow from the high-level dike compartments to the Pearl Harbor basal lens. Demands upon the Pearl Harbor aquifer will continue to increase with pumpage from the renovated HBWS Ewa Shaft, and future development requirements for the Hoopili, Koa Ridge, and Waiawa residential projects, along with the increased home basing of military troops. The latter will require the extended utilization of groundwater resources from among present Department of Defense sources—Waiawa Shaft, Red Hill Shaft, and the Halawa Shaft. Climate change effects on the groundwater flow regime, from dike compartments to basal lens, will escalate many competing demands. A greater understanding of the relationship among the windward high-level groundwater body and the Pearl Harbor basal lens would directly address these future concerns.

This study evaluates the relationship among the high-level windward dike compartment systems and the groundwater basal system of the Pearl Harbor aquifer. The hydrologic mechanisms of the movement of high-level groundwater to the basal lens are governed by two theoretical principles—laminar flow Darcy theory within the high-level dike compartments, and the Ghyben-Herzberg theory within the Pearl Harbor basal lens. Shallow index monitor wells in the basal lens (Fig. 2) would indicate the earliest temporal responses to groundwater movement—specifically direct recharge, originating from dike compartments

Through assessment of the relationship between the high-level groundwater dike compartments under prior drought conditions, and its attendant impact upon the Pearl Harbor basal lens, a proxy of future adverse climate change impacts can be identified. The results of the study are expected to assist policy makers in their preparations for climate change adaptive strategies. Identifying the temporal dynamics of the dike compartment and basal lens interaction may allow

sufficient preparation time for policy makers to program specific capital projects that address adverse climate change consequences.

The study is specifically aimed at evaluating the relationship among rainfall distribution, tunnel discharges, and potential adverse influences upon Pearl Harbor groundwater head levels because of climate change. The impacts upon recharge will not be quantified, but will be proxied by the response of Pearl Harbor index monitor wellhead-levels to the dynamic adjustments resultant from drought conditions. The scope of work will include literature review, data compilation, statistical analysis, monitoring of gauges, development-tunnel discharge measurements, and the evaluation of currently measured Waiahole Ditch discharge rates.

## **Methodology**

A review of the literature and the compilation of period of record data from the inception of the Waiahole Ditch System to the present are being conducted. The data includes precipitation records, Waiahole Ditch System discharge and instrument stage records, HBWS pumpage, deep monitor index well head level records, USGS Pearl Harbor spring discharges, and pumpage data from the Department of Defense and other sources. The study comprises data compilation, Waiahole Ditch System discharge measurements, and the application of statistical methods to assess the research variables. Climate data are used to assess the effects of previous episodic drought conditions upon the discharges of the Waiahole development tunnels, with the perspective of foreseeing sustainability impacts to the Pearl Harbor aquifer that may be attributable to adverse future climate-change conditions.

During the reporting period, pressure and barometric dataloggers were installed at Kahana Tunnel bulkheads A and B, Waihee Tunnel, and Kahaluu Tunnel (Fig. 3). A raingage was installed at the helipad of the Kahana Tunnel. Current-meter measurements are conducted at Kahana Tunnel, Waikane Tunnel 1, Waikane Tunnel 2, Uwau Tunnel, Release point 23, Release point 30, Release point 31, North Portal, and Adit 8, for comparison with the provisionally collected measurements submitted to Commission on Water Resource Management. These measurements will allow for an evaluation of the accountability of the relied-upon installed measuring devices and their attendant dataloggers. In tandem with these field measurements, the installation of dataloggers to track the Kahana Tunnel bulkhead-compartment pressure changes are incorporated. Compared against the measured stage heights of discharge flows of other Waiahole Ditch System development tunnels, the Kahana Tunnel bulkhead pressure will accurately represent dynamic dike-storage compartment changes.

In the next phase of the study, univariate multiple regression will be used to determine appropriate models that best represent the associations among the research variables. Semi-partial correlations will be assessed to determine its efficacy in isolating unique contributions of a particular predictor variable to a response variable. Contrast coding and other statistical methods may also be applied to identify completely independent contributions among predictor variables.

## Principal Findings and Significance

Examples of data compiled are shown in Figure 4, which illustrates index well trends and Waihee levels. Variability in head levels should be attributable to irrigation draft, direct recharge, and Waiahole Ditch influence upon Pearl Harbor recharge. In the early 1990s, the results seem to be influenced by sugar plantation-era draft. Figure 5 illustrates production of various system development tunnels and rainfall data compiled at the Waikane raingage. The figure reflects a relatively strong drought period in the 1940s that affected production of various tunnels. The data in the figure would facilitate calibration of a model and evaluating the Waiahole production based on various climate and land use scenarios. Figure 6 depicts the time variation of total tunnel production. A slight but consistent decline in production can be seen.

Figures 6 and 7 correlate production and rainfall data at two sites. The figures show consistent decline in production starting in the 1950s. It will be of interest to correlate the data various factors affecting the discharges. Figure 8 illustrates the difference between Waianu 29 and System Balance to Uwau Tunnel. The overall results indicate a reasonable water balance, although significant deviations exist.

Table 1 lists estimated long-term average production/discharge rates at various sites along the Waiahole system. Such estimates are useful for management purposes. However, variability around the averages and the expected effects of climate change should be also taken into account.

Table 1. Estimated long-term average production/discharge rates at various sites.

Site	Average production (+) or discharge (-) in mgd
Kahana Tunnel	+ 0.82
Waikane Tunnel 1	+ 4.78
Kahane 21	- 2.03
Release Point 23	- 3.23
Waikane Tunnel 2	+ 0.99
Uwau Tunnel	+ 13.88
Waianu 29	- 15.62
Gate 30	- 4.58
Gate 31	- 9.85
North Portal	- 5.24
Adit 8	+ 9.96

## **Publications Cited in Synopsis**

Takasaki, K.J., and J.F. Mink, 1985, Evaluation of major dike-impounded ground-water reservoirs, island of Oahu, U.S. Geological Survey Water Supply Paper 2217.



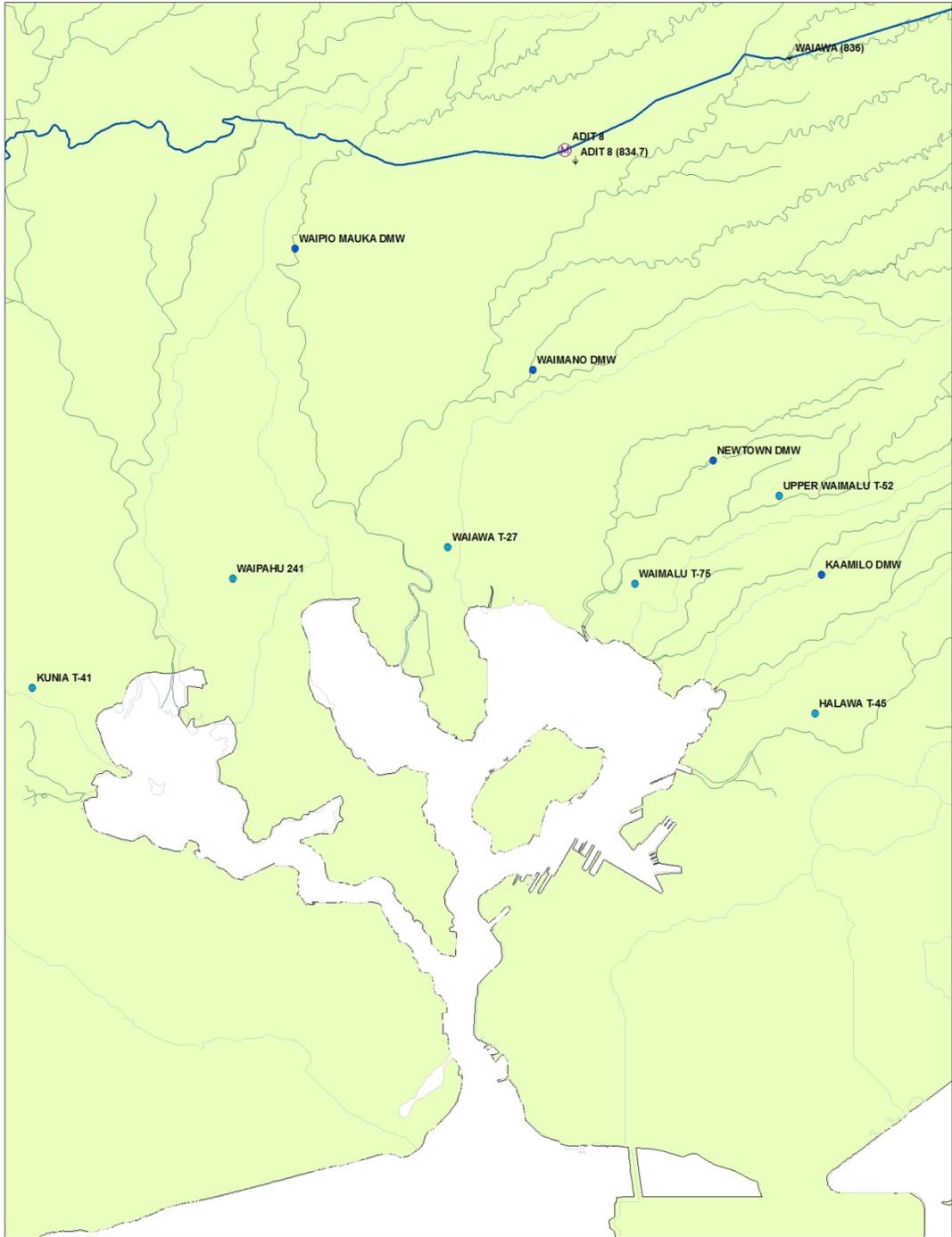


Figure 2. Pearl Harbor monitor wells.

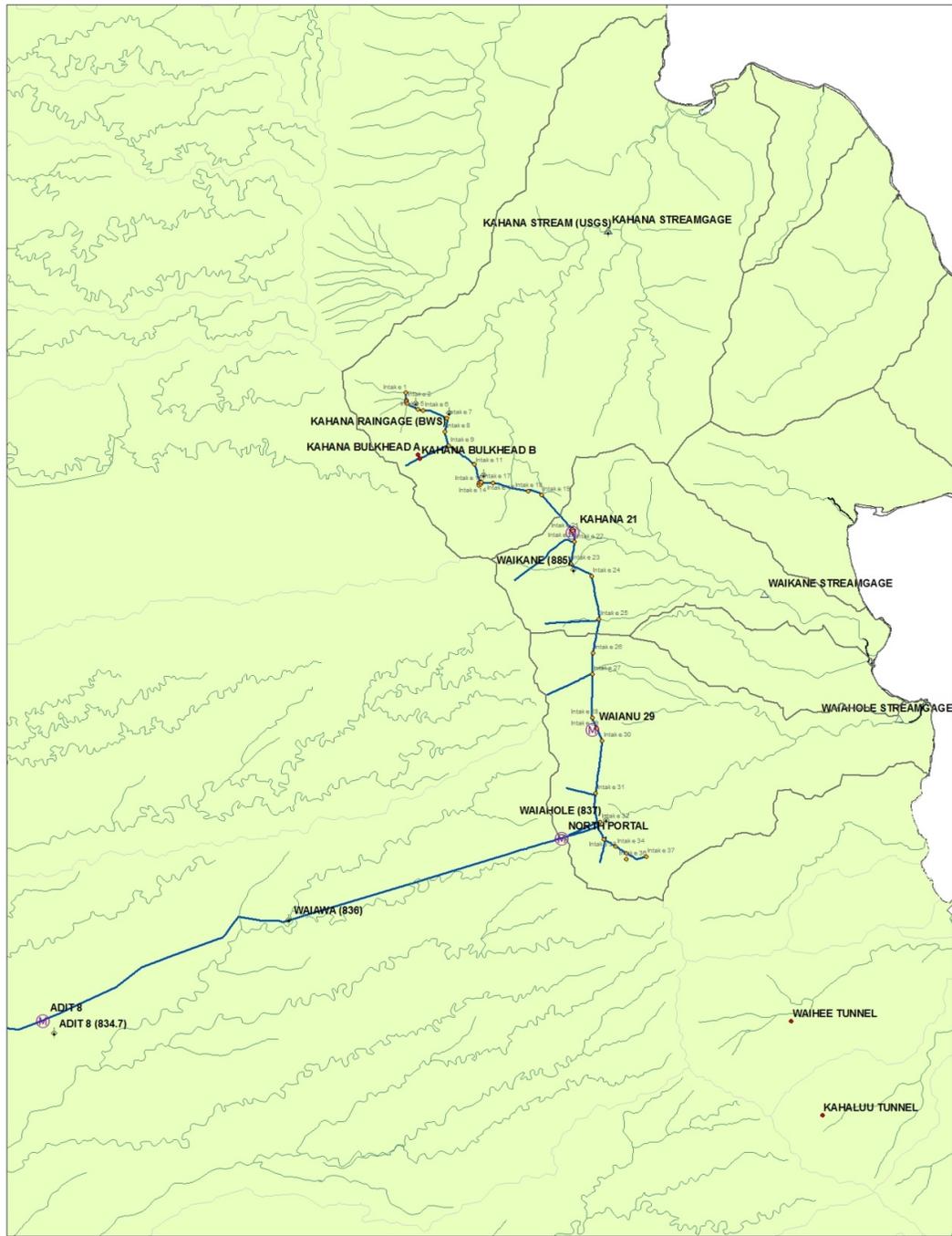


Figure 3. Data collection locations.

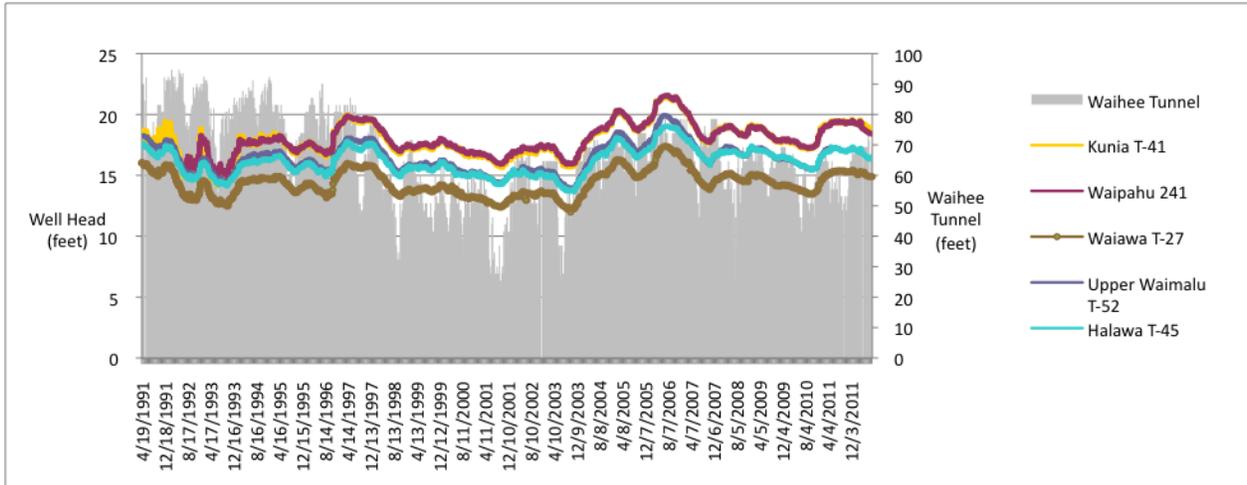


Figure 4. Pearl Harbor and Waihee Tunnel Head Levels. Locations in the Pearl Harbor are shown in Figure 2.

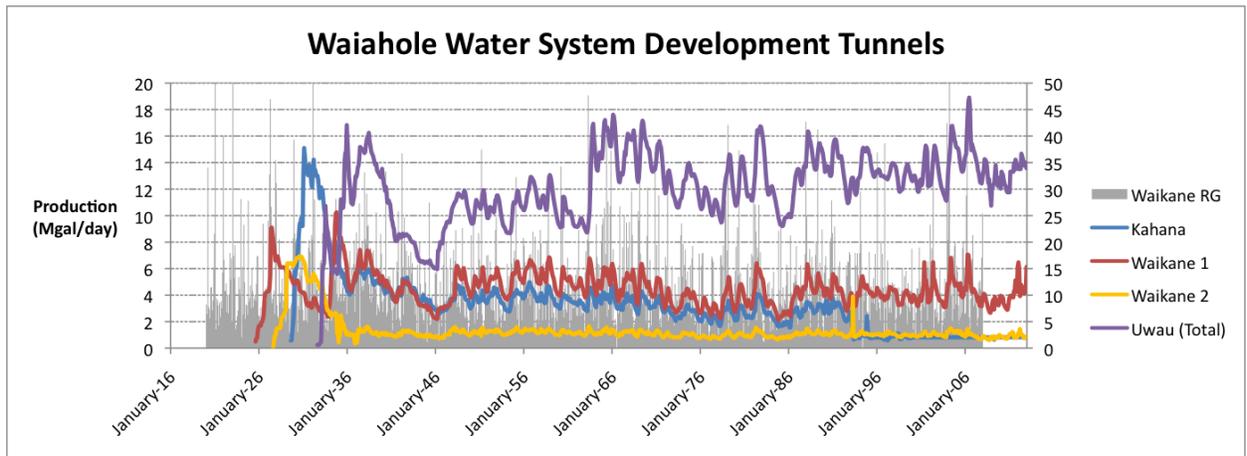


Figure 4. Data Compiled for the Waiahole Water System Development Tunnels (the right y-axis is for the Waikane Rain Gage data, inches).

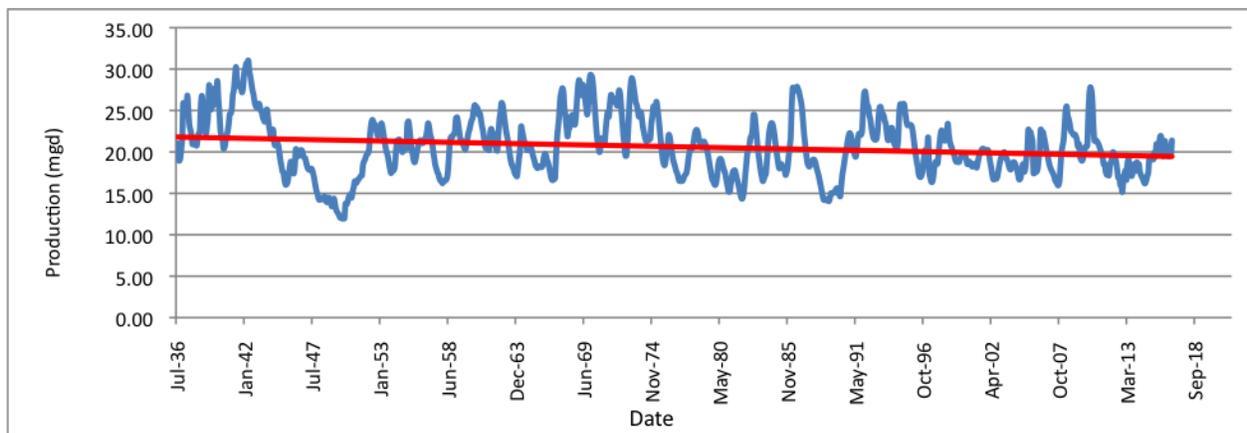


Figure 5. Total Tunnel Production. Linear trend line is also shown.

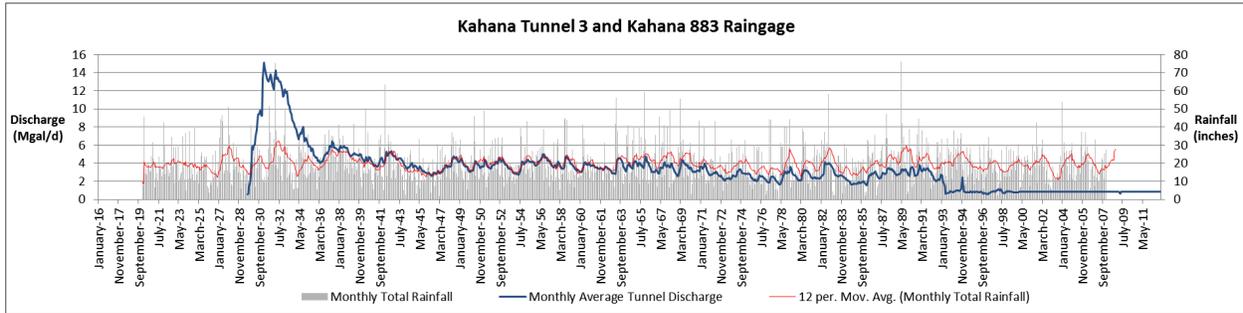


Figure 6. Discharge (mgd) at Kahana Tunnel 3 vs. rain data at Kahana 833 (inches).

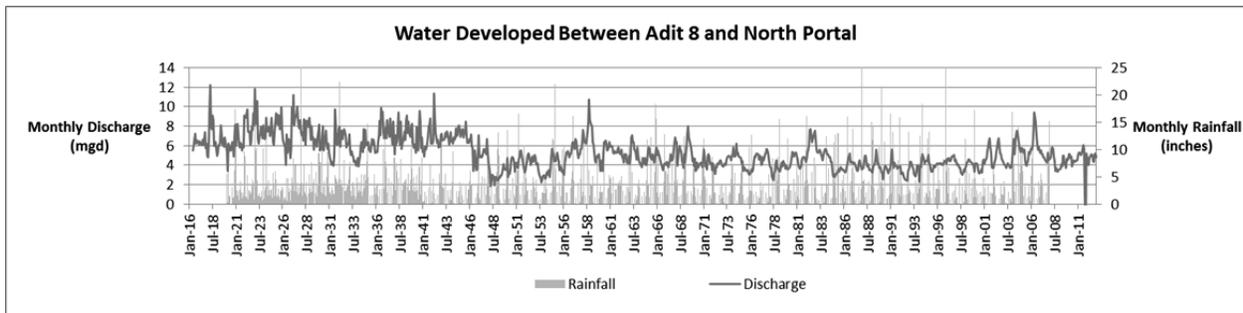


Figure 7. Water developed (mgd) between Adit 8 and North Portal vs. rainfall data (inches).

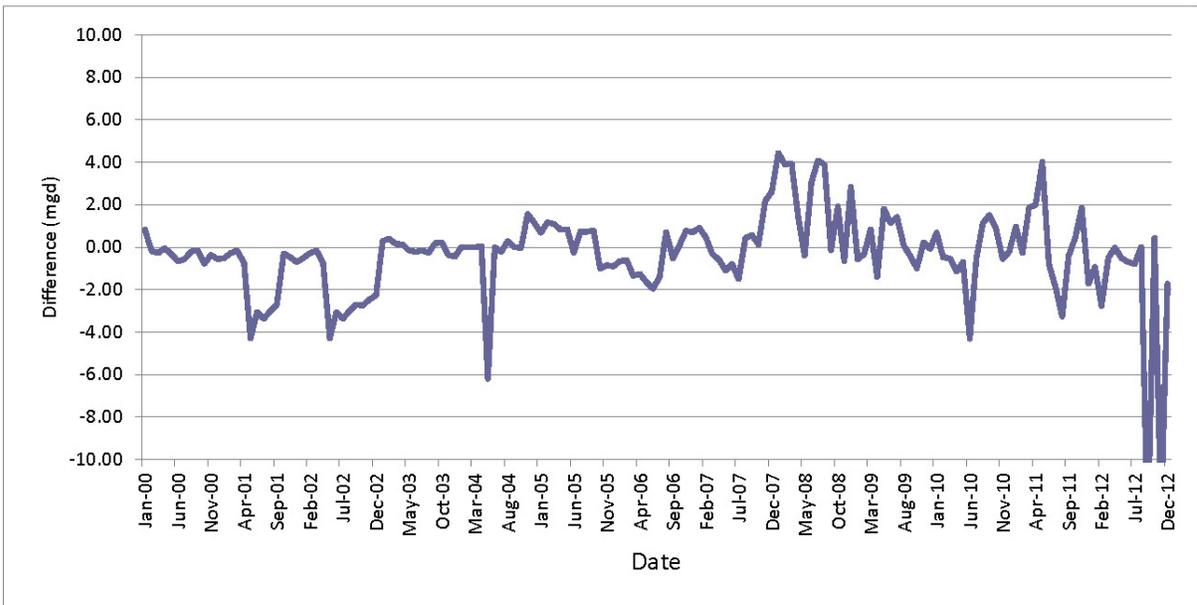


Figure 8. Difference between Waianu 29 and System Balance to Uwau Tunnel.

# Evaluation of next-generation sequencing technologies for environmental monitoring: characterization of microbial communities from wastewater plants and selected beaches of Hawaii using a metagenomics approach

## Basic Information

<b>Title:</b>	Evaluation of next-generation sequencing technologies for environmental monitoring: characterization of microbial communities from wastewater plants and selected beaches of Hawaii using a metagenomics approach
<b>Project Number:</b>	2013HI417B
<b>Start Date:</b>	3/1/2013
<b>End Date:</b>	2/28/2014
<b>Funding Source:</b>	104B
<b>Congressional District:</b>	1
<b>Research Category:</b>	Biological Sciences
<b>Focus Category:</b>	Water Quality, Methods, Ecology
<b>Descriptors:</b>	None
<b>Principal Investigators:</b>	Marek Kirs, Veljo Kisand

## Publication

1. There are no publications.

## **Problem and Research Objectives**

Microbes are the most abundant and diverse group of organisms on earth and major drivers of biogeochemical cycles in environments. Knowledge of the structure of an entire microbial community is important in understanding its function especially when the community comes under stress. Any kind of change in the surrounding environment will alter microbial communities. But the effect of subtle changes over a period of time (climate change, a small, but persistent contamination event) may not be seen when monitoring a single species of bacteria.

Coastal environments are highly complex and diverse. This diversity is maintained by niche availability and disturbances such as periodic terrestrial inputs or resuspension of sediment during storm events. These short term fluctuations may mask long term changes, but both have important implications on the environmental health of coastal waters and their use by the public. Knowledge of the coastal microbial community would give insights into the biodiversity and health of the communities and be helpful in the management of this resource.

Molecular methods have been the key tool to study microbial communities as most of the microbial flora is too challenging to be cultivated. Simple cloning, DGGE, TRFLP, PFGE and other related techniques have enhanced our understanding of microbial diversity, however, these methods provide only limited information about the microbial community structure and its function as only a limited number of individuals can be identified using these methods. Novel next-generation sequencing technologies, such as pyrosequencing, sequencing-by-synthesis and others, allow us to explore the microbial community structure in unprecedented detail as millions of sequencing reads are generated for each sample. Extremely detailed taxonomic and genetic profiles can be identified for each sample because the whole metagenome (genetic material present in the sample) is being analyzed. Furthermore, when targeting messenger RNA, this methodology (metatranscriptomics) can identify active gene profiles and provide information on the microbial community functions in the environment. For those reasons, next-generation sequencing has become a major tool to study the structural and functional changes of microbial communities (Kisand et al. 2012). Thus, wastewater and coastal microbial communities in Hawaii have not been studied using this approach hence we have limited understanding of microbial communities in our backyard.

To maintain a sustainable economy and healthy environment, decisions guiding environmental management need to be based on a broad and comprehensive understanding of the biodiversity and functional capability within the ecosystem (Kisand et al. 2012). Current monitoring programs target a few microbial indicators (such as enterococci) and therefore provide limited understanding of the ecosystem health. It is well established that anthropogenic contamination leads to reduce biodiversity (Vaughan et al. 2001), but this aspect is not targeted by current microbial monitoring programs. Strong evidence exists that environmental health and public health are tightly coupled. However, currently there is little research exploring the relationship between the effect of environmental change on microbial communities, microbial water quality, and public health. A more holistic approach is needed to fully understand the impact of coastal development and environmental change on both environmental and public health.

The research objectives are:

1. Provide in depth microbial community analysis for four wastewater treatment plants (WWTP) in Hawaii using next-generation sequencing tools,
2. Provide in depth microbial community analysis of selected beaches of Hawaii using next-generation sequencing tools,
3. To identify if and how wastewater treatment affect microbial communities in the wastewater discharge,
4. Identify microbial fingerprints associated with each major WWTP ocean discharge in Hawaii,
5. Establish an *in silico* data analysis pipeline for metagenomic research at WRRC, and
6. Establish an international collaboration with metagenomics experts at Tartu University, Estonia.

## Methodology

Water samples were collected from the following four wastewater treatment plants on Oahu: Honouliuli (09/2013), Kailua (10/2013), Sand Island (05/2013), and Hawaii Kai (10/2013) (Fig.1). Triplicate samples were collected three times within a single week from the raw influent, after primary treatment, after secondary treatment (when available), and from effluent at each WWTP. Coastal samples were collected along the Sand Island and Waikiki transect at sites routinely monitored by the City and County of Honolulu (CCH), which were in addition to the six sites around the Sand Island outfall (at three different depths with the assistance of CCH) (Fig. 2). Additionally, three sets of samples were collected for the analyses from the Manoa watershed (nine sites). Sample collection is complete.

After collection, samples were cooled on ice, transported to the laboratory where they were filtered through Supor 200 filters (0.2  $\mu\text{m}$  pore diameter) within 2 hours from collection, and stored at  $-40^{\circ}\text{C}$ . Triplicate filters were prepared from each sample. A total of 231 filters were stored at  $-40^{\circ}\text{C}$  for community analyses.

Concentrations of cultivable bacteria were identified from samples collected at Sand Island WWTP, around Sand Island outfall, and selected coastal samples on marine as well as on freshwater and marine water based R2A media. Over 1,000 colonies were isolated from the plates and stored at  $-20^{\circ}\text{C}$ .

Enterococci concentrations will be identified using standard membrane filtration technique on mEI media (Method 1600; USEPA 2002). Standard physical-chemical parameters (salinity, turbidity, pH) were also determined.

Sample collection is complete.

Samples will be extracted, amplified and sequenced for summer 2014. We will follow sequencing library preparation protocols outlined in Caporaso et al. (2012). The analysis of the data is scheduled for fall 2014, and the final report will be submitted March 2015.

Sequencing pricing and options have been negotiated with several service providers such as: Michigan University, Duke University, Yale University, University of Rhode Island, and Beckman Coulter.

## **Principal Findings and Significance**

We have completed our sample collection and cultivated the bacteria.

Concentrations of cultivable bacteria identified from the samples collected at the Sand Island WWTP, those surrounding the outfall, and from the coastal samples collected, are summarized in Table 1. There was good agreement between the enterococci concentration estimates identified by CCH and WRRC scientists. High concentration of enterococci was identified in the samples collected from shoreline S8 and offshore C5 surface. It remains to be seen whether the parallel sequencing tools will enable us to find the source of the elevated enterococci concentration.

Procedures for the analyses of the data have already been established at the WRRC Environmental Microbiology Laboratory and will be ready for the analyses of the sequencing data.

As stated in the introduction, the major goal of this project is to establish a research program to bridge environmental and public health sciences by leveraging novel community analyses tools to target environmental and human health concerns collectively, therefore providing a more holistic view of the coastal ecosystem and promoting sustainable development.

## **Publications Cited in Synopsis**

- Caporaso, J.G., C.L. Lauber, W.A. Walters, D. Berg-Lyons, J. Huntley, N. Fierer, S.M. Owens, J. Betley, L. Fraser, M. Bauer, N. Gormley, J.A. Gilbert, G. Smith, and R. Knight, 2012, Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms, *The ISME Journal*, 6(8), 162–1624.
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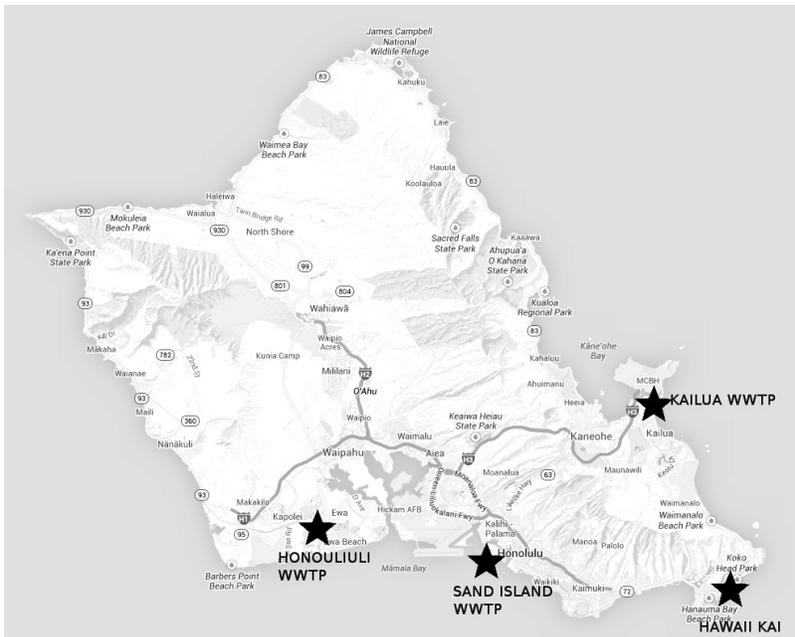
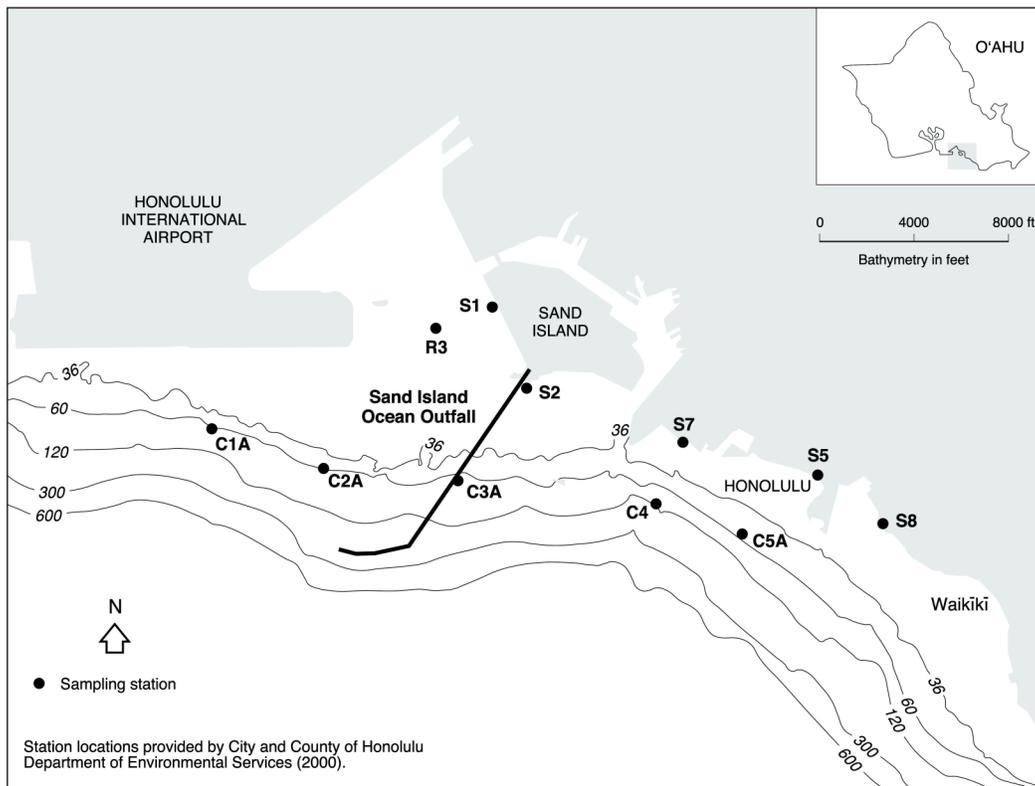


Figure 1 Wastewater treatment plants sampled in this study, Oahu, Hawaii.



Note: Sampling sites C = offshore, R = Keehi Lagoon, and S = shoreline. Sites C and R were sampled at 3 different depths.

Figure 2. Sampling sites surrounding the Sand Island ocean outfall, Oahu, Hawaii.

Table 1. Bacterial concentrations in the samples collected at the Sand Island WWTP. Sampling sites surrounding the outfall: C = offshore, R = Keehi Lagoon, and S = shoreline. Enterococci concentrations were determined by the City and County of Honolulu (CCH) and WRRC simultaneously. Note: The gray color indicates samples biased by overgrowth (yeasts and others); NT = not tested.

Sand Island WWTP	Salinity ppt	Enterococci CCH (mEI) CFU/100 ml	Enterococci WRRC (mEI) CFU/100 ml	R2A (Freshwater)			R2A (Marine)			Marine Agar		
				DAY 1 CFU/100 µl	DAY 2 CFU/100 µl	DAY 3 CFU/100 µl	DAY 1 CFU/100 µl	DAY 2 CFU/100 µl	DAY 3 CFU/100 µl	DAY 1 CFU/100 µl	DAY 2 CFU/100 µl	DAY 3 CFU/100 µl
Influent	5.8	NT	230,000	220,000	280,000	1,440,000	20,000	120,000	140,000	60,000	490,000	600,000
preUV	5.8	NT	17,000	90,000	450,000	780,000	0	50,000	80,000	20,000	110,000	110,000
Effluent	6.1	NT	1,100	0	150,000	190,000	10,000	30,000	30,000	0	60,000	270,000
C1S	33.6	0.9	1	0	0	0	8	72	73	3	9	53
C1M	33.5	<0.9	<1	0	0	0	4	141	129	2	43	110
C1B	33.6	<0.9	<1	0	1	3	16	187	165	2	5	44
C2S	33.6	<0.9	NT	0	1	2	5	12	24	1	31	63
C2M	33.5	<0.9	<1	0	0	6	4	142	264	7	18	35
C2B	33.7	<0.9	1	0	0	1	5	151	154	2	5	14
C3S	33.7	<0.9	<1	1	1	0	5	90	144	1	3	139
C3M	33.8	<0.9	<1	1	1	6	6	78	108	2	3	168
C3B	33.8	<0.9	<1	1	1	0	4	135	206	16	16	125
C4S	33.7	<0.9	<1	0	0	47	0	146	166	4	8	210
C4M	33.8	<0.9	<1	0	0	9	4	66	93	1	7	112
C4B	33.8	<0.9	<1	0	0	11	3	45	70	3	14	95
C5S	33.6	100	154	11	27	0	3	3	8	29	42	112
C5M	33.7	5	10	1	5	9	1	72	98	4	5	14
C5B	33.7	36	36	3	9	4	5	36	35	9	18	26
R3S	33.6	2	4	0	0	144	2	15	35	9	13	19
R3M	33.7	7	3	0	2	78	5	20	31	18	20	32
R3B	33.7	2	7	0	2	19	1	11	29	18	21	31
S1	33.3	3	13	1	8	12	30	66	60	24	34	26
S2	33.2	0.9	<1	0	4	6	33	55	52	13	40	15
S5	33.5	3	28	1	6	18	59	120	120	94	147	50
S7	33.5	9	4	0	0	0	9	46	63	34	54	54
S8	33.4	100	107	8	60	61	98	268	440	168	172	80

# Engineering plant peroxidases for wastewater treatment and water pollution monitoring

## Basic Information

<b>Title:</b>	Engineering plant peroxidases for wastewater treatment and water pollution monitoring
<b>Project Number:</b>	2013HI419B
<b>Start Date:</b>	3/1/2013
<b>End Date:</b>	2/28/2014
<b>Funding Source:</b>	104B
<b>Congressional District:</b>	1
<b>Research Category:</b>	Biological Sciences
<b>Focus Category:</b>	Methods, Wastewater, Treatment
<b>Descriptors:</b>	None
<b>Principal Investigators:</b>	Qing X Li, Eunsung Kan

## Publications

1. Zhao, H., T. Pan, Q.X. Li. Engineering highly stable palm tree peroxidase for phenol-containing waste water treatment. (in preparation)
2. Zhao, H., S. Li, Q.X. Li. Highly stable engineered peroxidase decorated Co/C core/shell nanoparticles: a robust and recyclable nanobiocatalyst for green synthesis of conducting polymer. (in preparation)
3. Pan, T., 2014, "Engineered palm peroxidase for use in treatment of BPA-containing wastewater," to be presented at Honors Fall Forum, University of Hawaii at Manoa, Honolulu, HI, December.

FINAL REPORT

**Engineering Palm Peroxidases for  
Wastewater Treatment and Water Pollution Monitoring**

**May 2014**

Qing X. Li  
Hongwei Zhao  
Tenny Pan  
Eunsung Kan

Project Number: 2013HI419B

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

## Abstract

Peroxidases are a large family of enzymes that typically catalyze an oxidation reaction utilizing peroxides such as hydrogen peroxide. Windmill palm peroxidase (WPP) is one of the most stable peroxidases, which possesses ultrahigh thermal stability (up to 90 °C) and extraordinary pH tolerance (pH 2 to 11). In this project, the peroxidase gene was cloned from palm tree leaves. Eleven mutations to the native palm peroxidase gene were designed based on the glycosylation sites of the native peroxidase and then created. The genes were transformed into yeast (*Pichia pastoris*) for expression. Of the eleven mutations, two yeast mutant strains produced a peroxidase that was highly active and stable. The engineered palm peroxidase has a His-Tag at the C-terminus of the amino acid sequence, which facilitates the palm peroxidase purification. The engineered peroxidase was purified and immobilized by Ni<sup>2+</sup>-nitrilotriacetic acid (NTA) derivative functionalized carbon coated cobalt magnetic nanoparticles (Co/C NPs). We designed and manufactured two new custom-made bioreactors. Each bioreactor has a pH probe, a DO probe, a temperature probe, and a motor for automatic stirring. Utilization of the bioreactor for palm peroxidase expression is being undertaken.

## Introduction

Peroxidases are a large family of enzymes that typically catalyze oxidation utilizing peroxides such as hydrogen peroxide. Horseradish peroxidase (HRP) is an enzyme widely used in water pollution monitoring and wastewater treatment. However, its poor stability has limited its applications, particularly for wastewater treatment. Many research efforts have been focused on searching for a HRP substitute. It has been demonstrated that palm peroxidase has exceptional stability, high activity and unique catalytic properties (Sakharov et al. 2001).

In this project, yeast (*Pichia pastoris*) was transformed with engineered genes to produce a novel palm peroxidase. Eleven mutations to the native palm peroxidase gene were designed based on the glycosylation sites of the native peroxidase and then created. Mutations at glycosylation sites determine the importance of the site for enzyme stability and activity. If the glycosylation site is deleted or edited, and the peroxidase functions normally, it can be assumed that the enzyme size can be made smaller through gene engineering with no loss of activity and possible increase in productivity. Another significance of enzyme size reduction is to improve wastewater treatment because enzymes small in size have high mass transfer and can improve wastewater treatment efficiency.

## Objectives

- 1) To obtain an enzyme mutant with a smaller size than the native.
- 2) To design a suitable bioreactor for enhancing engineered palm peroxidase productivity.

## Results and Discussion

The palm peroxidase gene sequence was synthesized according to the *P. pastoris* codon usage bias, which encodes an  $\alpha$ -mating sequence at the N-terminus for extracellular expression and an extra amino acid sequence GGSGGSHHHHHH (His-Tag) at the C- or N-terminus for its purification and immobilization. The synthetic gene was constructed into pPink-HC vector (Invitrogen) and transformed to PichiaPink Strain (Invitrogen) to screen high expression strains.

Upon testing both terminals for use in the purification step of the engineered peroxidase, it was found that a His-Tag at the C-terminal of the enzyme yielded significantly higher purification results. Thus, the C-terminal His-Tag was utilized for the engineered peroxidase.

The engineered peroxidase was purified and immobilized by Ni<sup>2+</sup>-nitrilotriacetic acid (NTA) derivative functionalized carbon coated cobalt magnetic nanoparticles (Co/C NPs). The Co/C core/shell magnetic NPs are commercially available in Turbobeats (Zurich, Switzerland) and Sigma-Aldrich (St. Louis, MO).

In general, enzyme can be covalently or non-covalently immobilized on magnetic NPs to fabricate nanobiocatalyst (Wang et al. 2009 and 2011; Lee et al. 2008; Dyal et al. 2003; Lee et al. 2009; Shukoor et al. 2008). Covalent attachment usually enables enzyme stabilization during catalysis; however, this often results in compromised enzyme activity due to the potential damage of the enzyme active center (Wang et al. 2011). Although absorption and entrapment-based non-covalent attachments could keep enzyme activity free, enzyme leakage will happen during recycling use because of the weak binding forces (Lee et al. 2009; Shukoor et al. 2008). Alternatively, affinity immobilization is an excellent methodology, since it can retain enzyme conformation and activity and minimize enzyme loss (Lee et al. 2009). Currently, His-tagged enzymes can be easily engineered and produced and can also be purified using traditional affinity chromatography or by using magnetic nanoparticles functionalized with metal-IDA/NTA (Wang et al. 2011). In the present study, strong magnetic Co/C NPs were simply functionalized with NTA derivative and were used to purify and immobilize the engineered peroxidase in one pot from the raw yeast fermentation culture medium.

Table 1. Primers used for site mutations at the glycosylation sites.

Primer	Sequence
GM-1-F	GACCTTCAGATTGGTTTTTACCAAACCTCCTGTCCAACCTGCTG
GM-1-R	CAGCAGTTGGACAGGAAGTTTGGTAAAAACCAATCTGAAGGTC
GM-2-F	GTCGCTGCCGCATTTGCCCAAATTCTGGTATTGCACCTG
GM-2-R	CAGGTGCAATACCAGAATTTGGGCAAATGCGGCAGCGAC
GM-3-F	GTCTTGTGGATTCTACAGCACAAAATACCGCTGAGAAGGACGC
GM-3-R	GCGTCCTTCTCAGCGGTATTTGTGCTGTAGAATCCAACAAGAC
GM-4-F	CTGCTAACCTTGCCGGTCAAATCACCTACCAGGTCCCTAG
GM-4-R	CTAGGGACCTGGTAGGTGATTTGACCGGCAAGGTTAGCAG
GM-5-F	CACAAATTCCATCACCTTTGTTTCAGGCTACTCAGCTTATCAACAG
GM-5-R	CTGTTGATAAGCTGAGTAGCCTGAAACAAAGGTGATGGAATTTGTG
GM-6-F	CAGCTTATCAACAGTTTCGCTCAAAGACTTTGACAGCCGATGAG
GM-6-R	CTCATCGGCTGTCAAAGTCTTTGAGCGAAACTGTTGATAAGCTG
GM-7-F	CAAACAGATTGTACAACCTCCAGTCAACTAGTGGAATTGACCCAAC
GM-7-R	GTTGGGTCAATTCCACTAGTTGACTGGAAGTTGTACAATCTGTTTG
GM-8-F	CTTAGAAACACATGCCAGCTCAGTCTACTAGATTCACTCCTATTAC
GM-8-R	GTAATAGGAGTGAATCTAGTAGACTGAGCTGGGCATGTGTTTCTAAG
GM-9-F	GCCTTGTTACCGAAGCACAGCTTTCGCGAGCTGTAAAGC
GM-9-R	GCTTTAACAGCTGCGGAAAGCTGTGCTTCGGTAACCAAGGC
GM-10-F	GCTGTAAAGCTAACGCCATGCAGTTGACTGCATGGGCTTCC
GM-10-R	GGAAGCCCATGCAGTCAACTGCATGGCGTTAGCTTTAACAGC
GM-11-F	CTCAAGGAGAGATCAGAACTCAGTGTTCCGTCGTCAACTCAGG
GM-11-R	CCTGAGTTGACGACGGAACACTGAGTTCTGATCTCTCCTTGAG

Eleven pairs of specific primers (Table 1) were designed for glycosylation site mutation. Each mutant vector sequence was verified by gene sequencing. After transformation of the vector into the yeast, expression was induced by adding 0.5% methanol to the culture medium for all eleven strains. Small amounts of enzyme were produced from the eleven mutants to serve as a baseline. The enzymatic activity of the eleven mutants was observed to select the mutation(s) that would yield a comparatively higher activity. Of the eleven, two strains produced an enzyme that was highly active and stable (GM-3, GM-7).

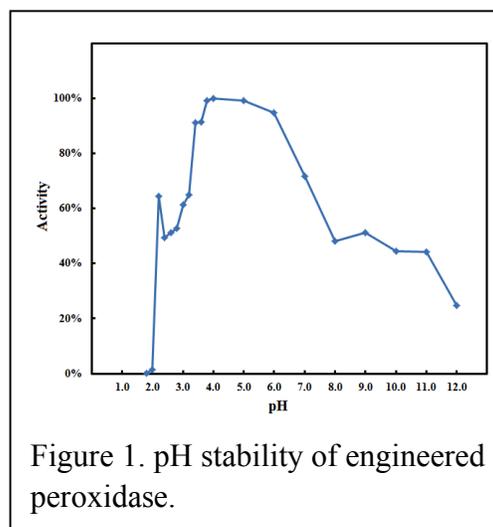


Figure 1. pH stability of engineered peroxidase.

The pH stability is important for engineered peroxidase for wastewater treatment. As shown in Figure 1, the engineered peroxidase was treated at different pHs (ranging from 2 to 12) for 1 h and was determined if the enzyme activity was compromised after treatment. The result showed that the engineered peroxidase can remain above 50% activity from pH 3 to 9, which is better than the HRP pH stability (pH 5 to 8).

The next step was to express large quantities of peroxidase using these two mutants. A rudimentary bioreactor was designed for this purpose (Fig. 2). This design was tested but yielded poor results. The monitoring of pH and O<sub>2</sub> levels, which is very important to maintaining a stable environment for the yeast growth and peroxidase expression, was nonexistent. With this design, the parameters were unable to be optimized, leading to a lower output than anticipated. Due to the lack of productivity with the initial bioreactor, we have designed and custom made a new bioreactor (Fig. 3). The new bioreactor (Fig. 3) is being utilized for the scaled-up expression. As shown in Figure 3, two identical bioreactors were designed and produced to facilitate the optimization of the enzyme expression parameters. Each bioreactor contains a pH probe, DO probe, temperature probe, and a motor for automatic stirring. Due to some parts of the bioreactor being unable to be autoclaved, whole bioreactor sterilization is currently being undertaken.

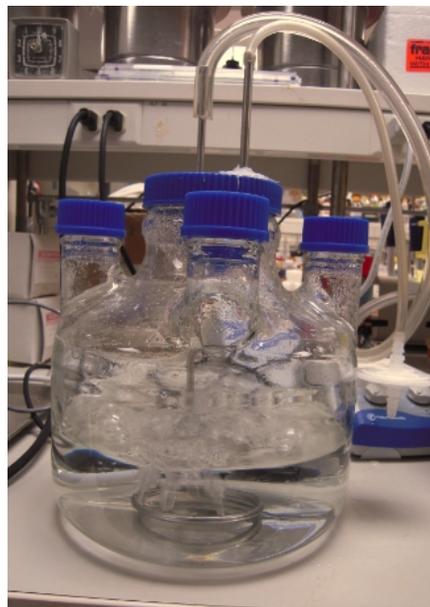


Figure 2. 5-L rudimentary bioreactor with air input.

### Notable Achievements

- Designed and manufactured two new custom-made bioreactors.
- Designed and created eleven mutants—two mutants (GM-3, GM-7) can produce active and stable peroxidase.
- Trained one undergraduate student.
- Trained one post-doctoral fellow. The post-doctoral fellow has been offered a new post as an associate professor.

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# Forecasting climate change impacts on watershed-based ecosystem services in Hawaii: A participatory modeling approach

## Basic Information

<b>Title:</b>	Forecasting climate change impacts on watershed-based ecosystem services in Hawaii: A participatory modeling approach
<b>Project Number:</b>	2013HI421B
<b>Start Date:</b>	3/1/2013
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<b>Funding Source:</b>	104B
<b>Congressional District:</b>	2
<b>Research Category:</b>	Social Sciences
<b>Focus Category:</b>	Methods, Models, Climatological Processes
<b>Descriptors:</b>	None
<b>Principal Investigators:</b>	Steven A. Gray, Ali Fares, Christopher A Lepczyk

## Publications

1. Htun, H., S. Gray, C. Lepczyk, K. Adams, S. Conant, and A. Titmus, Predicting Local Scale Climate Change Impacts on Endangered Birds by Combining Watershed Models with Expert Knowledge-based Modeling, Ecological Applications. (in preparation)
2. Gray, S.A., 2013, Decision-support Software for Federal Natural Resource Management Agencies, US Fish and Wildlife and National Park Service webinar series.
3. Htun, H., S. Gray, C. Lepczyk, A. Titmus, and S. Conant, 2013, "Predicting Local Scale Climate Change Impacts on Endangered Birds by Combining Watershed Models with Expert Knowledge-based Modeling for Decision-support," in 39th Albert L. Tester Memorial Symposium, Honolulu, HI.
4. Htun, H., S.G. Gray, C. Lepczyk, A. Titmus, and S. Conant, 2013, "Coupling of AnnAGNPS and Fuzzy-logic Cognitive Mapping to Facilitate Endangered Species Management Schemes Under Climate Change Scenarios," 2013 Hawaiian Wetlands and Waterbird Workshop, Kaneohe, HI.
5. Htun, H., S.G. Gray, C. Lepczyk, A. Titmus, K. Adams, and S. Conant, 2014, "Predicting Local Scale Climate Change Impacts on Endangered Birds by Integrating Watershed Models and Expert Knowledge-based Models for Decision-support," International Congress on Environmental Modelling and Software (iEMSs) 2014, San Diego, CA.
6. Htun, H., S.G. Gray, C. Lepczyk, A. Titmus, K. Adams, and S. Conant, 2014, "Predicting Local Scale Climate Change Impacts on Endangered Birds by Integrating Watershed Models and Expert Knowledge-based Models for Decision-support," in 2014 ESA Annual Meeting, Sacramento, CA.

## **Introduction**

The local-scale ecological impacts of global climate change are highly uncertain (Denman et al. 2007; Friedlingstein et al. 2006; Visser et al. 2000). Even though it is widely acknowledged that the impacts of these changes will be felt on the local level, modeling frameworks that translate these global changes in terms relevant to small-scale ecological decision-makers and natural resource managers remain somewhat elusive given the difficulties in down-scaling and coupling highly complex global processes with highly complex local processes (Denman et al. 2007; Friedlingstein et al. 2006; Sitch et al. 2008). The inability of current modeling methods to make climate change scenarios relevant at the local ecological scale emerges from a general lack of methods that allow resource managers to capture and link these global processes with local-scale dynamics that define the locally relevant connections between climate change, ecological dynamics and natural resource management priorities. Understanding relevant local scale dynamics and providing a way for local decision-makers to anticipate climate change impacts in terms relevant to their management priorities is therefore key if communities are expected to learn about and collectively adapt to undesired outcomes (Pahl-Wastl and Hare 2004).

## **Problem and Research Objectives**

Climate change in the Pacific Islands provide an ideal context to test ideas regarding the integrating of climate change scenarios, environmental data and expert-based model to understand and predict impacts. Climate change and its macro-effects on temperature, rainfall, sea level, and extreme events are likely to bring about localized changes in the coastal zone, agricultural systems, human settlements and infrastructure, water resources, human health, and macroeconomic performance in Pacific Island countries (Barnett 2005; Carter et al. 2001; Easterling et al. 2007). However, local-scale ecological dynamic changes brought about by changes in global carbon cycling are currently poorly understood (Denman et al. 2007; Friedlingstein et al. 2006; Sitch et al. 2008) although some general trends have recently emerged (Rosenzweig et al. 2007). For example, recent studies suggest that there will be less frequent but higher intensity rainfall patterns across the Pacific Islands due to climate change. Decreases in precipitation and base flow (Chu et al. 2010; Oki 2004), the continuation of these decreasing trends may impact freshwater ecosystems and aquatic species. Decrease in streamflow (Bassiouni and Oki 2012; Oki 2004) may also interrupt movement of native species along streams and may prevent species that spend their larval stages in the ocean from returning to the streams to complete their life cycle (Keener et al. 2012). Invasive plant and animal species are established and expanding in many forests and their responses to climate change will interact with those of native species to determine future ecosystem composition and processes. Native species have to compete with alien species for food and shelter. Existing climate zones are projected to shift, generally upslope, with some eventually disappearing (Benning et al. 2002). The ability of native plant species to adapt to these changes will be affected by competition with aggressive invasion. Available habitat decreases rapidly with elevation, putting species currently found on upper slopes and ridges at special risk (Eiben and Rubinoff 2010; Keener et al. 2012). Currently, native species in their most pristine conditions are located in these regions with high elevation. Spread of invasive species to these regions is a foreseeable risk caused by climate change.

Although researchers are beginning to synthesize the ecological impacts of these effects cumulatively (Price et al. 2009), information about how habitat managers can mitigate these unwanted outcomes is generally not available. Indeed, climate change has already affected Hawaii by increasing air and sea-surface temperatures that exceed the global average (Giambelluca et al. 2008) and a 10% reduction in streamflow over the last 30 years (Oki 2004). General circulation model projections indicate that continued warming and drying will be paired with more intense, yet less frequent, rainfall events (Chu and Chen 2005; Chu et al. 2010; Norton et al. 2011) and stream flow in some Hawaiian watersheds will continue to be reduced by 6.7% up to 17.2% over the next 40 years (Safeeq and Fares 2011). In addition, erosion regimes and changes in nutrient loading are expected to accompany changes in water availability (Furniss et al. 2010). All of these hydrological and ecological changes to Hawaii's watersheds are thought to be accompanied by decreases in habitat suitability for native species (Keener et al. 2012). However, methods to forecast future changes in terms useful for wildlife management are highly underdeveloped and poorly understood, especially on island communities that are predicted to be disproportionately affected by climate change (Barnett 2005). Insufficient and uncertain comprehension of climate change induced outcomes is thought to limit social adaptation responses (Pahl-Wastl and Hare 2004). Therefore, unique island hydrogeology and climate, together with ambiguous climate change prediction, will add uncertainties and complications to climate change management planning. While it is unknown how watershed and habitat decision-making in the future will be affected by climate changes, poorly-informed decision-making and failure to reduce uncertainty associated with these changes will likely increase the social and ecological costs of climate change on island communities.

Given the difficulties in understanding how water quality and quantity changes will impact habitat, we present a modeling approach intended to provide decision-support by coupling watershed data generated through common models with a relatively novel method of pooling expert knowledge to predict how watershed changes will impact life history functions of managed bird species, mediated by understanding changes in habitat dynamics. To demonstrate the usefulness of this approach to understanding complex habitat changes based on climate change projections, in this paper, we use case study data collected from three endangered water birds (Hawaiian Stilt, Coot, and Moorhen) on the Hawaiian Island of Kauai.

## **Methodology**

The modeling framework includes integrating two models in a five-stage process: (1) defining climate change scenarios, (2) modeling watershed changes under climate change scenarios using AnnAGNPS, (3) describing relationships between watershed dynamics and bird habitat using expert-based modeling using Fuzzy-logic Cognitive Mapping (FCM), (4) developing model scenarios by integrating the two models, and (5) using scenario results to discuss adaptation and management strategies.

## Climate Change Scenarios

To define climate change reference scenarios we use data from the IPCC report that includes different CO<sub>2</sub> emission rates and their effect on temperature and precipitation changes for the next 100 years. Four CO<sub>2</sub> emission rates (330 ppm [2003–2004 rate], 550 ppm, 710 ppm, and 970 ppm) were used for developing model scenarios. In addition, precipitation changes ( $\pm 5\%$ ,  $\pm 10\%$ , and  $\pm 20\%$ ) specific to the Hawaiian Islands were also used for scenarios based on previous work (Safaeq and Fares 2011). The model simulations were based on the IPCC's extreme temperature values of the "likely" range (1.1°C and 6.4°C). Safaeq and Fares (2011) previously developed 24 scenarios in the Pacific Islands using these three components (CO<sub>2</sub> emission rates, temperature, and precipitation variations), however, only six climate change scenarios (extreme as well as intermediate scenarios) out of these twenty-four were selected as reference models, which reduced the complexity of the full combination of all possible scenarios into those that were represented the most variation.

## Watershed Model—AnnAGNPS

Empirical data available from different sources were used as model input for the watershed model, which included: digital elevation model map, soil, rainfall, annual isohyets, land cover, temperature, sky cover, wind speed/direction, and evapotranspiration calculated using FAO method. These data were organized as input files for AnnAGNPS simulation. AnnAGNPS simulated sediment and nutrients due to primary sources of pollutants found in the Hanalei Watershed and they are feral ungulates and alien plants that increase erosion in the upper watershed (increased suspended solids, nutrients, and pathogens), cesspools and septic systems in urban areas, agricultural operations (taro ponds), water bird impoundments, and cattle grazing.

## Fuzzy-logic Cognitive Mapping

To develop the ecological model based on the knowledge held by local scientific experts and couple it with the climate and watershed model output, we used a FCM program called Mental Modeler (see [www.mentalmodeler.org](http://www.mentalmodeler.org)) (Gray et al. 2013). To construct the conceptual expert model, we held a participatory modeling workshop with four local ecologists who were experts in the three species being modeled followed by two individual meetings with scientific experts to refine the model. One FCM was developed for each of the three endemic species that defined relationships between life-history with habitat variables and variables from the watershed models and predator/prey interactions. As a reference for model building, the workshop used Kauai National Wildlife Management Guidelines and a literature search for each of the three species, watershed variables, predator/prey dynamics, and other relevant factors important.

Fuzzy-logic Cognitive Mapping with the Mental Modeler software includes semi-quantitative models of environmental issues and these models are developed through a three-step process by (1) defining the important system components relevant to an individual or community, (2) identifying the strength of relationships between component pairs, and (3) simulating scenarios on these models to determine how the system as a whole might react under a range of possible

conditions (i.e., system state changes). System components are physical or environmental aspects and may include (1) attributes or characteristics, (2) elements, or (3) processes of a system or place. They are interconnected to represent complex, direct, and indirect interactions or relationships with one another. Relationships between system components indicate the way one component influences or affects another (such as directionality, strength of the relationship, and type of relationship). Relationship directionality is represented in the FCMs as arrows that start at one component and end at another. The relationship arrows may be one-way (uni-directional) or both directions (bi-directional). The strength of the relationship between components may be classified as High (H, strong), Medium (M, moderate), or Low (L, weak), defined either positively or negatively. Positive effects mean that the component the relationship arrow originates from increases the other component. Negative or inverse relationships mean that the component that the relationship arrow originates from decreases the other component. This effect is represented by a plus (+) or a minus (–) symbol following the H, M, or L strength designation (Gray et al. 2013).

## **Principal Findings and Significance**

Model results based on IPCC scenarios suggest that increased precipitation will increase Stilt abundance, but decrease Coot and Moorhen abundance. On the other hand, decreasing precipitation may have similar effects across all three species. Additionally, our integrated model scenarios suggests that climate change scenarios will impact life functions (e.g., parental success, breeding, foraging success, etc.) differently across the three species. Combining empirical and expert-based conceptual models allows managers to understand the local ecological impacts associated with global climate change, making it relevant to the management scale. Additionally we suggest this framework can be easily employed by wildlife managers to understand the impacts of climate change on different types of animals and across different ecological conditions.

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# Quantifying Toxoplasmosis gondii presence in wastewater and freshwater systems

## Basic Information

<b>Title:</b>	Quantifying Toxoplasmosis gondii presence in wastewater and freshwater systems
<b>Project Number:</b>	2013HI422B
<b>Start Date:</b>	3/1/2013
<b>End Date:</b>	2/28/2015
<b>Funding Source:</b>	104B
<b>Congressional District:</b>	1
<b>Research Category:</b>	Water Quality
<b>Focus Category:</b>	Water Quality, Acid Deposition, Acid Deposition
<b>Descriptors:</b>	
<b>Principal Investigators:</b>	Christopher A Lepczyk

## Publication

1. There are no publications.

## **Introduction**

*Toxoplasma gondii* is a parasitic protozoan that is shed from the body via oocysts in cat feces and is often consumed by other animal species living on land and in the water. In Hawaii, toxoplasmosis has been found post mortem in terrestrial and marine animals, including endangered species (e.g., Hawaiian Crow and Red-footed Boobies, Monk Seal: Dawson 2010; Honnold et al. 2005; Work et al. 2000, 2002). Similarly, off California, toxoplasmosis has been associated with mortality in sea otters (Jessup et al. 2007). In humans it can cause serious effects on the unborn, pregnant women, and immunocompromised individuals (Torrey and Yolken 2003). Furthermore, toxoplasmosis causes subtle changes in behavior, especially for assessing risk, and has been linked to schizophrenia (Torrey and Yolken 2003; McAllister 2005; Lafferty 2006).

Cats, including house cats (*Felis catus*), are the definitive host, meaning that the disease only occurs when cats are present. Toxoplasmosis transmission has traditionally been associated with eating uncooked meat or soil, but it can also be acquired by inhalation from dust, soil, or cat litter, and from water contaminated by cat feces (Benenson et al. 1982; Dabritz et al. 2006; Duffy 2012). This project will provide needed information on the public health and environmental risks associated with this waste product in Hawaiian freshwater systems. Quantifying levels of toxoplasmosis in aquatic systems is of great interest to state and federal agencies, including the State of Hawaii's Department of Land and Natural Resources, State of Hawaii's Department of Health, the U.S. Fish and Wildlife Service, and the National Oceanic and Atmospheric Administration, due to both the human and animal health problems associated with the parasite.

## **Problem and Research Objectives**

Because Hawaii has a large number of free roaming cats there is a large potential for fecal matter to enter the water system both via overland flow and through the sewage system, as occurs in California (Dabritz et al. 2006). Given the human and environmental health concerns associated with Toxoplasmosis, our objective is to determine its presence and relative abundance in the waste disposal system of Honolulu and freshwater systems of Oahu. By testing both freshwater streams and wastewater plant streams we will be able to provide the first ever assessment of toxoplasmosis in Hawaii's aquatic systems.

## **Methods**

In determining if toxoplasmosis is present in Oahu streams the first step is conducting water sampling for the parasite. In the first year of the project several water filtering approaches have been investigated based on sampling equipment, cost, and ability to effectively capture oocysts. At present different filtering methods are being screened. Following water sampling the samples will be filtered for collection of oocysts. The elute from the filter will be analyzed with PCR against known markers for toxoplasmosis. Similar work has already been done on fecal samples and yielded positive results.

## Principal Findings and Significance

In progress, nothing to report.

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# Impacts of Climate Change on Food Resources of Native Gobiid Fishes and Atyid Shrimp in Hawaiian Streams

## Basic Information

<b>Title:</b>	Impacts of Climate Change on Food Resources of Native Gobiid Fishes and Atyid Shrimp in Hawaiian Streams
<b>Project Number:</b>	2013HI423B
<b>Start Date:</b>	3/1/2013
<b>End Date:</b>	2/28/2015
<b>Funding Source:</b>	104B
<b>Congressional District:</b>	2
<b>Research Category:</b>	Biological Sciences
<b>Focus Category:</b>	Ecology, Hydrology, Climatological Processes
<b>Descriptors:</b>	None
<b>Principal Investigators:</b>	Rebecca Ostertag, Richard Ames MacKenzie

## Publications

1. Riney, M.H., T.C. Frauendorf, R.A. MacKenzie, P.B. Foulk, and R. Ostertag, 2013, "Using Stable Isotopes and Gut Content Analysis to Assess the Impacts of Climate Change on Food Webs in Hawaiian Streams" in Society for Freshwater Science Conference, Jacksonville, FL.
2. Riney, M.H., T.C. Frauendorf, R.A. MacKenzie, P.B. Foulk, and R. Ostertag, 2013, "The Impacts of Climate Change on Food Webs in Hawaiian Streams" in Tropical Conservation Biology and Environmental Science Symposium, University of Hawaii at Hilo, HI.
3. Riney, M.H., planned December 2014, "The Impacts of Climate Change on Food Webs in Hawaiian Streams," M.S. Thesis, Tropical Conservation Biology and Environmental Science, University of Hawaii at Hilo, HI. (in preparation)
4. Riney, M.H., T.C. Frauendorf, R.A. MacKenzie, R. Tingley III, P.B. Foulk, and R. Ostertag, The Effects of Varied Stream Flow on Food Webs in Hawaiian Streams, Journal of the Society for Freshwater Science. (in preparation)

## **Progress**

This project is designed to accomplish the following objectives: 1) provide a food web analysis based on two critical native faunal species in Hawaiian streams along a naturally occurring precipitation gradient; 2) quantify effects of flow rates on food resource availability for both species; 3) determine if resource limitation is leading to interspecific competition; and 4) provide data on effects of altered flow rates due to climate change, invasive species, and water diversions, that will help assist in conservation and restoration efforts.

To meet these four objectives, the field work and the sampling of invertebrates and the food resources was completed by August 2013. The native gobiid fish were excluded due to permitting constraints, and non-native caddisflies were substituted as a proxy for the food web. All samples were analyzed by a mass spectrometer for stable carbon and nitrogen isotope signatures by April 2014. Preliminary data analysis was completed by April 2014 and will be finalized by August 2014.

Preliminary results point to possible resource partitioning between male and female atyid shrimp in high flow streams, but not in low flow streams. In the high flow stream, the male atyid shrimp appear to be feeding on filamentous algae while the female shrimp appear to be feeding on biofilm. In the low flow stream, both the males and the females appear to be feeding on biofilm.

## **Student Support**

Graduate student, Michael Riney, is currently in the data analysis stage and will be submitting his M.S. thesis prior to graduating in December 2014. He has given two oral presentations based on his research through this project: “Hawaii Ecosystems Conference, July 2013” and “Tropical Conservation Biology and Environmental Science Conference, April 2014”.

## Monthly rainfall maps for the islands of Kauai, Oahu, Molokai, Maui, and Hawaii, 2008-2010

### Basic Information

<b>Title:</b>	Monthly rainfall maps for the islands of Kauai, Oahu, Molokai, Maui, and Hawaii, 2008-2010
<b>Project Number:</b>	2013HI437S
<b>Start Date:</b>	6/17/2013
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<b>Funding Source:</b>	104B
<b>Congressional District:</b>	
<b>Research Category:</b>	Climate and Hydrologic Processes
<b>Focus Category:</b>	Climatological Processes, Hydrology, None
<b>Descriptors:</b>	
<b>Principal Investigators:</b>	Thomas Giambelluca

### Publication

1. There are no publications.

## **Introduction**

The Hawaiian Islands have one of the most diverse rainfall patterns on earth. The mountainous terrain, persistent trade winds, heating and cooling of the land, and the regular presence of a stable atmospheric layer at an elevation of around 7,000 ft interact to produce areas of uplift in distinct spatial patterns anchored to the topography. The resulting clouds and rainfall produced by this uplift lead to dramatic differences in mean rainfall over short distances. Knowledge of the rainfall patterns is critically important for a variety of resource management issues, including groundwater and surface-water development and protection, controlling and eradicating invasive species, protecting and restoring native ecosystems, and planning for the effects of global warming.

## **Problem and Research Objectives**

The Rainfall Atlas of Hawaii is a set of maps of the spatial patterns of rainfall for the major Hawaiian Islands (Giambelluca et al. 2013). The maps represent the best estimates of the rainfall for the 30-yr period 1978–2007. In recent years, it has become apparent that monthly maps for the period 2008 to 2010 are needed for numerous water-resource investigations being undertaken by Federal, State, and County agencies in Hawaii. The updated maps are needed to extend the available rainfall record through 2010 to enable analyses of more recent hydrological conditions in Hawaii.

Monthly rainfall maps are foundational products needed to develop water budgets and estimates of groundwater recharge. Understanding changes in the spatial distribution of rainfall is critical for the protection and management of water resources in Hawaii. Hawaii's small land masses and isolation limits its water-resource options, making assessment of water availability even more critical to Federal, State, and local decision makers.

The objective of this project is to develop monthly rainfall maps for 2008 through 2010 for the islands of Kauai, Oahu, Molokai, Lanai, Maui, and Hawaii using an approach that was previously used for the Rainfall Atlas of Hawaii.

## **Methodology**

The objective for this project will be met using well-documented methods (Giambelluca et al. 2013) to produce rainfall maps in a manner consistent with methods previously used for the Rainfall Atlas of Hawaii. This will enable meaningful temporal and spatial comparisons to be made of rainfall in Hawaii. Spatial datasets will be constructed using the same grid layout and map projection as was used for the Rainfall Atlas of Hawaii, and will be provided in the same digital format. Rainfall maps will be provided as completed.

## **Principal Findings and Significance**

All rainfall station data from over 10 networks has been collected, formatted, and quality controlled (including standardizing units so all networks could be combined into one file). Data for 2007 were also collected so the new station data can be matched with previous records. Many networks use unique identifier numbers for their stations, so work has been done to match these stations with standardized State Key Numbers (SKNs). Most of the data from these networks were available online for downloading and processing, however, some data from the State Climatologist office had to be hand entered. These data were entered twice so that the two datasets could be compared and errors removed. One of the islands' recent data was not available in the State Climatologist office, and therefore had to be located and hand entered. For new stations (not previously recognized by the State Climatologist), new SKNs have been assigned. A master list of the currently operating stations, along with their metadata, has been compiled.

## **Publications Cited in Synopsis**

Giambelluca, T.W., Q. Chen, A.G. Frazier, J.P. Price, Y.-L. Chen, P.-S. Chu, J.K. Eischeid, and D.M. Delporte, 2013: Online Rainfall Atlas of Hawai'i. *Bull. Amer. Meteor. Soc.* 94, 313–316, doi: 10.1175/BAMS-D-11-00228.1.

# Assessment of Hydraulic Properties through Tidal Ground-level Analysis for the State of Hawaii

## Basic Information

<b>Title:</b>	Assessment of Hydraulic Properties through Tidal Ground-level Analysis for the State of Hawaii
<b>Project Number:</b>	2013HI438S
<b>Start Date:</b>	8/5/2013
<b>End Date:</b>	12/31/2014
<b>Funding Source:</b>	104B
<b>Congressional District:</b>	1
<b>Research Category:</b>	Ground-water Flow and Transport
<b>Focus Category:</b>	Groundwater, Models, None
<b>Descriptors:</b>	
<b>Principal Investigators:</b>	Aly I El-Kadi

## Publications

1. Rotzoll, K., D.S. Oki, and A.I. El-Kadi, 2014, Development of a hydrogeologic framework from tidal analysis of coastal groundwater levels, Hawaii, 40th American Water Works Association Conference, Honolulu, May 6–9.
2. Rotzoll, K., D.S. Oki, and A.I. El-Kadi, 2013, Development of a hydrogeologic framework using tidally influenced groundwater levels, Hawaii. Abstract H11H–1248, presented at 2013 Fall Meeting, AGU, San Francisco, CA, Dec. 9–13.

## **Introduction**

The Hawaii aquifers supply water to 1.36 million residents, diverse industries, and a large component of the U.S. military in the Pacific. With the increase of resident population on the islands, drinking water resources remain limited and are therefore susceptible to impacts from human activity and climate change (Izuka 2013). Decisions related to future infrastructure development and alternate sources of freshwater, including desalinization, depend on the long-term sustainability of the groundwater resources in the Hawaii aquifers. Models that are used to manage groundwater availability and quality require a good understanding of the hydrogeologic framework of the aquifers and accurate estimates of hydraulic properties to simulate groundwater flow and evaluate saltwater intrusion.

The ocean-tide signal attenuates through coastal aquifers based on the aquifer's regional hydraulic diffusivity (Jacob 1950; Ferris 1951). Thus, analyzing tidal amplitude decay with distance from the coastline allows estimating regional-scale hydraulic properties and identifying the importance of different hydrologic units (Rotzoll et al. 2013). A few localized studies of tidally influenced groundwater levels in Hawaii exist (e.g., Dale 1974; Gingerich 1995; Oki 1997; Rotzoll et al. 2008), but a statewide compilation and comparison is lacking.

Numerical groundwater models are becoming standard tools for sustainable development and optimal resource management (e.g., Oki 2005; Gingerich 2008). Identifying relevant components in the hydrogeologic framework and quantifying hydraulic properties for volcanic rock aquifers helps to constrain input parameters for models used in ongoing U.S. Geological Survey (USGS) groundwater-availability and groundwater-resources studies in Hawaii (Izuka 2013). Additionally, evaluating hydraulic properties for coastal sediment aquifers can be beneficial for studies assessing the effects of sea-level rise, e.g., groundwater inundation, that is including groundwater height in coastal inundation scenarios (Rotzoll and Fletcher 2013).

The aquifer properties were examined in a regional context with respect to the hydrogeologic framework of each island. Hydrologic data exist for some areas in Hawaii, but there has been no effort to synthesize the existing separate hydrogeologic frameworks into a comprehensive framework for the entire Hawaiian Islands region. The results help to better understand the groundwater flow processes in Hawaii aquifers and facilitate the development of regional numerical groundwater flow and transport models. Water managers and hydrologists benefit from a more detailed characterization of the regional groundwater-flow properties for various purposes (e.g., monitoring, management, research).

## **Problem and Research Objectives**

The scope of work examines the large-scale estimation of hydraulic properties of volcanic-rock and coastal-sediment aquifers from tidal attenuation by compiling available data for the state of Hawaii.

## Methodology

Tidal attenuation is analyzed by comparing amplitudes of groundwater level fluctuations occurring on tidal frequencies against ocean tide amplitudes. Besides incorporating published tidal-attenuation data from Pearl Harbor, Oahu (Dale 1974), the North Shore of Oahu (Oki 1997), Honolulu, Oahu (Rotzoll and Fletcher 2013), east Maui (Gingerich 1995), and central Maui (Rotzoll et al. 2008), existing continuous water-level records were analyzed. Continuous ocean water level is recorded at several tide gages in Hawaii (National Oceanographic and Atmospheric Administration 2013). Groundwater level records are collected by the USGS and other entities in various wells across the state. Furthermore, unpublished hydrographs from USGS well folders are digitized and compared to historic tides. Although generally short in nature, tidally influenced constant-rate aquifer test data are digitized from state well-permit applications and compared to ocean tide amplitudes.

Filtering methods to separate water-level fluctuations caused by ocean tides and other environmental stresses, such as barometric pressure and long-period ocean level variations, are explored. For short-term records, several approaches to identify tidal components are examined.

The results of tidal influence are separated by island, if possible, by each volcano, and by volcanic rock and coastal sediment aquifers. The aim is to include areas that were previously not assessed comprehensively (e.g., Kauai, Molokai, and the Island of Hawaii). Moreover, groundwater records with little or no tidal influence in coastal areas allow identifying areas that have limited hydrological connection to the ocean. Tidally derived aquifer parameters were compared statewide.

## Principal Findings and Significance

Hawaii aquifers are enormously heterogeneous. However, they can be reduced to three simplified hydrogeologic units: (1) dike-intruded volcanic rock, (2) dike-free volcanic rock, and (3) sediments and rejuvenated Volcanics. The tidal response in groundwater levels can help identify these hydrogeologic units and aid in estimating regional hydraulic properties. For example, no tidal signal was observed in the hydrogeologic unit of dike-intruded volcanic rock, and the lower-permeability caprock unit causes a damping effect of the tidal signal at the boundary. The first assessment indicates that aquifer-diffusivity and hydraulic-conductivity estimates in dike-free volcanic rock decrease with island age, consistent with the reduction in permeability through weathering. The results will be used in regional island-wide numerical models through the USGS Groundwater Resources Program.

## Publications Cited in Synopsis

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- Oki, D.S., 1997, Modeling the effects of pumping, barometric pressure and ocean tides on ground-water levels in northern Oahu, Hawaii, Honolulu, Hawaii, University of Hawaii, Ph.D. dissertation, 321 p.
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- 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.
- Rotzoll, K., and C.H. Fletcher, 2013, Assessment of groundwater inundation as a consequence of sea-level rise, *Nature Climate Change*, 3(5), 477–481.
- 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*, 21(3), 643–654.

## **Information Transfer Program Introduction**

A small state like Hawaii allows many opportunities for researchers to interact with administrators and policy makers. In hopes of broadening knowledge of and appreciation for Hawaii's water resources, WRRC's technology transfer program produces newsletters, organizes and publicizes seminars, workshops and conferences, assists in producing posters and other materials for presentations, and maintains the center's website.

# Technology Transfer

## Basic Information

<b>Title:</b>	Technology Transfer
<b>Project Number:</b>	2013AS414B
<b>Start Date:</b>	3/1/2013
<b>End Date:</b>	2/28/2014
<b>Funding Source:</b>	104B
<b>Congressional District:</b>	1st
<b>Research Category:</b>	Not Applicable
<b>Focus Category:</b>	Education, None, None
<b>Descriptors:</b>	None
<b>Principal Investigators:</b>	Philip Moravcik

## Publications

There are no publications.

## **Introduction**

In 2013, the University of Hawaii Water Resources Research Center (WRRC) was given the added responsibility as the WRRIP Center for American Samoa. During the reporting period, we have established contact with representatives from the American Samoa Power Authority (ASPA), the agency that manages the main population Center's water system, the American Samoa Environmental Protection Agency (ASEAP), and the American Samoa Community College (ASCC). We currently have one research project underway in American Samoa having gotten a slow start due to the funding uncertainties of the WRRIP program last year. This ongoing project has generated a wealth of data that is currently under processing (see report of project 2013AS424B, Assessing Ground Water Sustainability of the Island of Tutuila, American Samoa). Extensive communications have been established with ASPA, ASEPA, and ASCC to identify areas of needed research beyond those covered in the project 2013AS424B. These include the need to explore the potential for developing alternate water supply sources in high-elevated groundwater zones. Data has been provided to ASPA for potential utilization in their planning and management activities. We have also developed a training program for ASCC students that involved coordinating with a faculty member at the College. The local group is helping in collecting project data, which has eliminated the need for our staff's repeated travel. The knowledge gained by the faculty member and the students will be of great benefit to their careers. WRRC's Advisory Council includes members from American Samoa and were heavily consulted before and during proposal development and during research evolution. The members participated in our Council last meeting through a phone conference and provided a list of research concerns for American Samoa.

Although the technology transfer program for American Samoa is still in the early stages, we expect that there will be considerable synergy between the programs in Hawaii and American Samoa. WRRC's director and Technology Transfer Specialist will be traveling to Samoa to conduct meetings and further develop a network of contacts sometime this year.

## **Problem and Research Objectives**

The mandate of WRRC 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.

## **Methodology**

The Technology Transfer Office employs a variety of techniques to disseminate the results of research done at the Center. The office transfers information concerning water resource research and issues to its audience through WRRC bulletins and other publications, the web site, workshops, meetings, conferences, and regular biweekly seminars. Other activities include providing information to consultants, students of all levels, and the public, and participating in school science fairs and in research projects. The staff also regularly updates and improves the Center's web site.

## **Technology Transfer Program Activities**

WRRC's Technology Transfer Program activities for American Samoa during the report period were aimed at to establishing dialogue and coordinating research plans and activities with our

American Samoan partners. The activities also included discussion of needed research and training of students. The communications were facilitated by WRRRC graduate student Christopher Schuler who has been spending time in Samoa working on the WRRIP research project “Assessing Ground Water Sustainability of the Island of Tutuila, American Samoa”.

Mr. Schuler recently presented a public seminar at the University of Hawaii about his project: Challenges to Groundwater Sustainability in Tutuila, American Samoa.

# Technology Transfer

## Basic Information

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

## Publications

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
2. Moravcik, Philip 2012, Water Resources Research Center - Researcher Profile - Interest Guide, internal publication of the University of Hawaii Water Resources Research Center, 1-20
3. Moravcik, Philip. 2013. Bulletin, Water Resources Research Center, University of Hawaii at Manoa (July), <http://www.wrrc.hawaii.edu/bulletins/2013/July2013bulletin.pdf>, 10 pages
4. Moravcik, Philip. 2013. A Brief Overview of Research Conducted at Hawaii's Water Resources Research Center, University of Hawaii at Manoa (July), <http://www.wrrc.hawaii.edu/WRRChistory.pdf>, 8 pages

## **Technology Transfer**

The mandate of the Water Resources Research Center (WRRC) 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.

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.

WRRCs 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 Centers web site.

Major activities conducted by the Technology Transfer Office included the following:

- Seminars

One of the principal activities of the Technology Transfer Office is the organization of the Center's seminar series.

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 Quality 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 Surface Water</i>	Dr. Rhonda Loh, Chief of Natural Resources Management, 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 (3<sup>rd</sup> 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 the 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 Advanced Biosensors Laboratory Center for Biological Defense Dept. of Cell Biology, Microbiology and Molecular Biology (CMMB), 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 &amp; Limits of Correlation with Microbial Indicators</i>	Pierre Payment, Professeur Centre INRS-Institut Armand-Frappier, Laval, Québec, Canada Institut national de la recherche scientifique
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](http://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 committee

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:

<b>Hawaii Water Resources Research Center Advisory Council 2012</b>	
<b>Name/Title</b>	<b>Agency</b>
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
<b>For American Samoa</b>	
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.

- 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.

# Technology Transfer

## Basic Information

<b>Title:</b>	Technology Transfer
<b>Project Number:</b>	2013HI413B
<b>Start Date:</b>	3/1/2013
<b>End Date:</b>	2/28/2015
<b>Funding Source:</b>	104B
<b>Congressional District:</b>	1st
<b>Research Category:</b>	Not Applicable
<b>Focus Category:</b>	Education, None, None
<b>Descriptors:</b>	None
<b>Principal Investigators:</b>	Philip Moravcik

## Publications

1. Moravcik, Philip. 2013. Bulletin, Water Resources Research Center, University of Hawaii at Manoa (July), 10 pp. <http://www.wrrc.hawaii.edu/bulletins/2013/July2013bulletin.pdf>
2. Moravcik, Philip. 2013. A Brief Overview of Research Conducted at Hawaii's Water Resources Research Center, University of Hawaii at Manoa (July), 8 pp. <http://www.wrrc.hawaii.edu/WRRChistory.pdf>

## **Introduction**

A small state like Hawaii allows many opportunities for researchers to interact with administrators and policy makers. In hopes of broadening knowledge of and appreciation for Hawaii's water resources, WRRC's technology transfer program produces newsletters, organizes and publicizes seminars, workshops and conferences, assists in producing posters and other materials for presentations, and maintains the center's website.

## **Problem and Research Objectives**

The mandate of the Water Resources Research Center (WRRC) 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.

## **Methodology**

The Technology Transfer Office employs a variety of techniques to disseminate the results of research done at the Center. The office transfers information concerning water resource research and issues to its audience through WRRC bulletins and other publications, the web site, workshops, meetings, conferences, and regular biweekly seminars. Other activities include providing information to consultants, students of all levels, and the public, and participating in school science fairs and in research projects. The staff also regularly updates and improves the Center's web site.

## **Technology Transfer Program Activities**

WRRC's Technology Transfer Program activities for the report period included: organization of multiple seminars; production of the Center newsletter; participation in meetings and conferences; and providing water-resources-research information to consultants, students of all levels, and the general public. The program PI also participated in school science fairs, research projects, and refinement of the center's web site. During the current reporting period the Technology Transfer Program produced one newsletter describing research projects and center activities and news. During this reporting period the Technology Transfer PI made extensive use of the center's large-format printer/plotter, producing posters for display at local, national, and international meetings and conferences. Two of these posters, illustrating the work of graduate student researchers, won awards at conferences.

## **Meetings**

The Technology Transfer Office helped to organize a meeting: U.S. Recreational Water Quality Criteria: A Vision for the Future held in Honolulu, March 11–13, 2013. The Hawaii Center has a long history of involvement in the examination and investigation of recreational water quality microbial standards and has organized several previous meetings of national experts.

In December 2011, USEPA published a draft of the proposed recreational water quality criteria. This draft document indicated that the revised Recreational Water Quality Criteria (RWQC) would not differ substantially from the previous recreational water quality criteria published in 1986. However, there was a significant change in this document in that the new guidelines allow individual states to use alternative methods to monitor water quality, and to implement state-specific water quality standards. The effectiveness of these new guidelines has not been tested at the state level. Moreover, during the planning of this conference, USEPA had not yet published the guidelines for alternative methods and standards, nor finalized the proposed criteria; hence the specifics of implementation strategies as well as incentives to develop and adapt alternative standards remained unclear. For these reasons WRRC felt that science-based assessment of RWQC and discussion on how new science can be used to take advantage of the flexibilities in the new RWQC were needed. The conference focused on the use of scientific method to achieve the following goals: Evaluate the methods and approaches that were used in developing and implementing the new RWQC. Evaluate how newer methods and approaches can be used to establish site-specific criteria. Evaluate how published experimental methods can be used to determine health risk to swimmers and be applied to develop RWQC. Invited conference speakers included some of the foremost experts in the field of recreational water quality standards. They were selected based on their acknowledged expertise on the topics chosen for this conference and their demonstrated use of scientific methods in water quality research and monitoring programs. In addition to the oral presentations there were over 30 poster presentations outlining the work being done at many academic and government departments about work being done to address the shortcomings of the current system of recreational water quality monitoring. Among other work, the Technology Transfer Office produced the website for the conference. An announcement for the conference can be viewed at [www.wrcc.hawaii.edu/\\*cleanup/rwqc2013/announce&prog.pdf](http://www.wrcc.hawaii.edu/*cleanup/rwqc2013/announce&prog.pdf).

In April WRRC hosted a workshop on advanced modeling of water flow and contaminant transport in porous media using the HYDRUS and HP1 software packages. HYDRUS is Windows-based modeling software that can be used for analysis of water flow, heat and solute transport in variably saturated porous media (e.g., soils). The HYDRUS suite of software is supported by an interactive graphics based interface for data-preprocessing, discretization of the soil profile, and graphic presentation of the results. HYDRUS has been used in hundreds, if not thousands of applications referenced in peer-reviewed journal articles and many technical reports. The software packages are also used in classrooms of many universities in courses covering soil physics, processes in the vadose zone, or vadose zone hydrology. The workshop covered a detailed conceptual and mathematical description of water flow and solute transport processes in the vadose zone. Hands-on computer sessions provided participants with an opportunity to become familiar with the software packages, including several additional modules, such as ROSETTA, HP1, UNSATCHEM, and/or the Wetlands module. Emphasis was on preparation of input data for a variety of one- and multi-dimensional applications. Advanced topics covered in the second part of the workshop included: coupled movement of water, vapor, and energy (including the surface energy balance), preferential/non-equilibrium water flow and solute transport (using dual-porosity and dual-permeability models), biogeochemical transport (using the UNSATCHEM and HP1 (coupled HYDRUS-1D and PHREEQC) modules), modeling flow and transport using a three dimensional module of HYDRUS (2D/3D)

The workshop instructor was Dr. Jirka Šimunek from the Department of Environmental Sciences, University of California Riverside, CA. This is the third HYDRUS workshop that WRRC has sponsored.

## Publication—A Brief Overview of Research Conducted at Hawaii’s Water Resources Research Center

In order to educate and inform a new generation of University administrators, water agency officials, consultants, and the public the WRRC’s Technology Transfer Specialist wrote and distributed an eight-page synopsis of the Center’s research activities over its 50-year history. This document provides a brief background of the WRRIP program and a concise description of the kinds of research that the Hawaii center has done over the years, categorizing that research broadly into fourteen focus areas. There have been many changes to the University of Hawaii administration as well as to the personnel at local water agencies in recent years and it was felt that it would be timely to inform the newcomers about the Center’s productive history.

## Seminars

The Technology Transfer Program organizes biweekly seminar series 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 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.

In response to numerous requests from individuals around our geographically isolated islands the Technology Transfer office undertook to video record and post the seminars on the internet to permit our neighbor island colleagues to benefit from our seminar presentations without the expense of travel to Honolulu. Links to the videos are now provided on the seminar listing page at the Center’s website every semester.

The following is a list of the seventeen seminars presented during the reporting period.

## FY 2013 Seminars

### Spring 2013 Coordinator Dr. David Penn

March 6, 2013	<i>Introducing the Genuine Progress Indicator (GPI) to Hawaii</i>	Regina Ostergaard-Klem, Hawaii Pacific University, College of Natural & Computational Sciences, Global Leadership & Sustainable Development and Kirsten Oleson, University of Hawaii.
March 14, 2013	<i>Who are we Overlooking in Microbial Diversity Surveys and Does it Really Matter?</i>	Mitchell Sogin, senior scientist and director of the Josephine Bay Paul Center for Comparative Molecular Biology and Evolution, Marine Biological Laboratory in Woods Hole, Mass.

March 20, 2013	<i>Recovering from a Legacy of Mining in Crested Butte</i>	Anthony Poponi, Former Director for the Coal Creek, CO Watershed Coalition and Standard Mine Technical Advisory Group.
April 10, 2013	<i>Numerical Modeling of Water Flow and Solute Transport in Soils Using HYDRUS including Preferential Flow, Colloid-Facilitated Contaminant Transport, and Various Biogeochemical Processes</i>	Jirka Simunek, Professor and Hydrologist, Department of Environmental Sciences, University of California Riverside.
April 17, 2013	<i>Surface-water Availability During Low-Flow Conditions: Case study in Anahola Stream, Kaua'i, and Hawai'i Statewide Application</i>	Chui Ling Cheng Hydrologist, U.S. Geological Survey Pacific Islands Water Science Center.
Apr 23, 2013	<i>Submerged Anaerobic Membrane Bioreactor for Low Strength Wastewater Treatment</i>	Ng, How Yong, Associate Professor, Centre for Water Research, Department of Civil & Environmental Engineering, National University of Singapore.
May 1, 2013	<i>Water Planning for the Manoa Campus, University of Hawaii (UHM)</i>	Sharon Ching Williams, UHM Campus Architect.

**Fall 2013 Coordinator Dr. Aly El-Kadi**

September 5, 2013	<i>Updates from the State of Hawaii Environmental Health Administration, State Commission on Water Resource Management, and Honolulu Board of Water Supply</i>	Gary Gill, Deputy Director, Environmental Health Administration, State of Hawaii Department of Health; Roy Hardy, Hydrology Program Manager, State Commission on Water Resource Management; Glenn H. Oyama, Hydrologist-Geologist, Honolulu Board of Water Supply.
September 19, 2013	<i>Conservation Agriculture Production Systems (CAPS): Building Resilience into Smallholder Agriculture</i>	Travis Idol, Professor, Natural Resources and Environmental Management, University of Hawaii at Manoa.
October 3, 2013	<i>Precipitation Variability and Trend in Hawaii</i>	Pao-Shin Chu, Professor and State Climatologist, Department of Meteorology, University of Hawaii at Manoa.
October 17, 2013	<i>Submarine Groundwater Discharge Links Watershed Hydrology and Coastal Biogeochemistry</i>	Henrieta Dulaiova, Assistant Professor, Department of Geology and Geophysics, University of Hawaii at Manoa.
November 7, 2013	<i>Island Hydrology: Time to Take a Closer (and Broader) Look at Confining Structures</i>	Don Thomas, Director, The Center for the Study of Active Volcanoes (CSAV), University of Hawaii at Hilo.

November 21, 2013	<i>Modeling Specific Groundwater Problems in Hawaii: Borehole Flow and Groundwater Inundation</i>	Kolja Rotzoll, Department of Geology and Geophysics, University of Hawaii.
December 5, 2013	<i>Integrating Demand- and Supply-Side Management: Pricing and Watershed Conservation</i>	Kimberly Burnett, Associate Specialist, University of Hawaii Economic Research Organization.

### Spring 2014 Coordinator Dr. Sayed Bateni

February 3, 2014	<i>The Value of an Innovative System to Process Large Volumes of Water (100 l) to Detect Microbial Pathogens in Sources of Water Used for Drinking, Recreational, Re-use, Agricultural and Food Production</i>	Daniel V. Lim, Ph.D.; Elizabeth A. Kearns, Ph.D., and Sonia Magaña, B.S., Advanced Biosensors Laboratory Department of Cell Biology, Microbiology, and Molecular Biology University of South Florida Tampa, Florida.
February 4, 2014	<i>Multi-Scale Laboratory Experimentation for Process Understanding and Up-Scaling for Field Problem Solution</i>	Tissa H. Illangasekare, AMAX Distinguished Chair of Civil and Environmental Engineering Director, Center for Experimental Study of Subsurface Environmental Processes Colorado School of Mines, Golden, Colorado, USA.
February 25, 2014	<i>Plans for World Wide Public Health Risk Assessment and Regulations for Hygienic Quality of Beach Sand and Beach Water</i>	João Carlos Simões Brandão, Medical Mycologist, Reference Unit for Parasitic and Fungal Infections - Department of Infectious Diseases, Instituto Nacional de Saúde/National Institute of Health.

### WRRC Website

The Technology Transfer Office continually updates the departmental website ([www.wrhc.hawaii.edu](http://www.wrhc.hawaii.edu)) with new information about WRRC researchers' activities, seminars, reports, meetings, grant announcements, and the Center's L. Stephen Lau scholarship fund. The site provides information about center facilities and personnel as well as a database of WRRC publications. A web-site search function provides easy access to the available information.

As mentioned above we are now recording and posting center seminars on the internet to permit distant colleagues the opportunity to see our seminars from their home islands. Links to these videos are provided on the seminar webpages. For example: [www.wrhc.hawaii.edu/spring2014seminars.shtml](http://www.wrhc.hawaii.edu/spring2014seminars.shtml). We have added an RSS feed to the home page ([www.wrhc.hawaii.edu](http://www.wrhc.hawaii.edu)) that aggregates water news from a variety of sources including the technology transfer offices own blog. This adds a more dynamic feel to the Center's website.

## **Digitization and Online Posting of Center Publications**

The technology transfer office continues to add publications to the University of Hawaii at Manoa's ScholarSpace institutional repository database. Several years ago the Technology Transfer Office initiated the digitization of the Center's large archive of reports generated by the many projects conducted at the Center over the past 40 years. These reports are available for downloading in PDF format at the ScholarSpace website (<http://scholarspace.manoa.hawaii.edu/>). Previously, individuals wanting to access the information in these reports would have to write to WRRC and request to have a copy mailed to them for a cost. This initiative represents a substantial milestone in our efforts to disseminate the results of our Center's research. 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. Three graduate-research-assistant posters were recognized by conference awards.

## **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.

## **Editing of Research Articles, Reports, Proposals**

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.

## **L. Stephen Lau Scholarship**

The Technology Transfer Office took responsibility again this year for coordinating the announcement, application review, applicant selection for the Center's 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.

## The Center's Advisory Council

The center convenes a council of local water stakeholders in Hawaii and Samoa in order to help guide our research priorities. No meeting of this group was held during the reporting period however we continue to maintain contact with the council members in anticipation of our next meeting.

### Members of the Center's Advisory Council include:

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
<b>For American Samoa</b>	
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

# USGS Summer Intern Program

None.

<b>Student Support</b>					
<b>Category</b>	<b>Section 104 Base Grant</b>	<b>Section 104 NCGP Award</b>	<b>NIWR-USGS Internship</b>	<b>Supplemental Awards</b>	<b>Total</b>
<b>Undergraduate</b>	2	0	0	1	3
<b>Masters</b>	2	0	0	0	2
<b>Ph.D.</b>	1	0	0	0	1
<b>Post-Doc.</b>	1	0	0	2	3
<b>Total</b>	6	0	0	3	9

## **Notable Awards and Achievements**

For 2013HI\_419B: a. Designed and manufactured two new custom-made bioreactors.

b. Designed and made 11 mutants, two mutants (GM-3, GM-7) can produce active and stable peroxidase.

# Publications from Prior Years