Challenges in microbial water quality studies in Hawaii



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What is the best indicator of clean beach water?

| This Factor BEST Explains What Clean Beach Water Means | Responding 'Yes' (%) |
|---|-------------------------|
| Clear or colorless water | 19.1 |
| No disease-causing pathogens | 48.7 |
| No Trash | 23.7 |
| No wildlife | 0.4 |
| Odorless water | 8.1 |

Jones, et al., 2018

Why water quality matters?



Economy

Globally: ~120 million cases of GI and > 50 Million cases of severe RD per year from swimming and bathing in polluted <u>coastal</u> waters (Shuval, 2003)
Loss: \$700 million per year (Shuval, 2005)
US: 90 million cases illnesses per year from <u>recreational water use</u>
Loss: \$2.2-3.7 billion per year (DeFlorio-Barker, et al.,2018)
(Illness based burden, environmental impact and related losses not accounted for)

How clean are our beaches?

Hawaii: Ranked Number 7th (out of 30) in the US

Hawaii 2013 Beach Water Quality Summary



Percent of samples exceeding the national Beach Action Value (BAV) safety threshold 415 beaches (88%) were not monitored or had a limited number of samples (fewer than 12) 20 beaches (4%) did not have any samples exceed the national BAV safety threshold 25 beaches (5%) had >0-10% of their samples exceed the national BAV safety threshold 4 beaches (1%) had >10-20% of their samples exceed the national BAV safety threshold 6 beaches (1%) had more than 20% of their samples exceed the national BAV safety threshold



% exceedance of national BAV safety threshold

* Please note exceedance rates for 2013 are shown based on the new BAV safety threshold and the historical national standard for comparison purposes. Additionally, only samples from a common set of beaches monitored each year from 2009-2013 are included in the bar chart.

(NRDC, 2014: "Testing the Waters")

What makes you sick?

Pathogenic bacteria, viruses (Wade et al., 2018), and protozoa (sewage-borne, environmental)

What illnesses?

- Gastroenteritis
- Skin rash
- Eye and ear infections
- Respiratory infections
- Wound infections
- Others...

How are the water quality standards derived? EPA epidemiological studies



Based on studies conducted on beaches impacted by point-source! Can be too protective when major source of indicator bacteria is non-point (HI)

Figs from: US EPA 2012. Recreational Water Quality Criteria

Regulations

EPA Recreational Water Quality Criteria (RWQC) (2012)

Health risk based:

Green recommendation – illness rate 36 per 1000 recreators

Red recommendation – illnesses rate 32 per 1000 recreators

| | Geometric Mean Density | Beach Action Value (BAV) 75 th percentile | Statistical Threshold Value (STV) 90 th percentile (10% samples should not exceed) |
|-------------|------------------------------|---|---|
| | | (MPN or CFU / 100 ml) | |
| Freshwater: | | | |
| Enterococci | 35 or 30 | 70 or <mark>60</mark> | 130 or 110 |
| E. coli | 126 or 100 | 235 or 190 | 410 or 320 |
| Marine: | | | |
| Enterococci | <u>35</u> or <mark>30</mark> | 70 or <mark>60</mark> | <u>130</u> or 110 |

IMPORTANT: health risk established on beaches impacted by point sources (sewage)

Regulations

EPA Recreational Water Quality Criteria (RWQC) (2012)

RWQC are used to:

1) derive water quality-based effluent limits (WQBELs) National Pollutant Discharge Elimination System (NPDES) permits,

2) identify impaired and threatened waters for waterbody assessments (§303(d) reporting),

3) develop waste load allocations and load allocations for TMDLs, and

4) for beach notification programs under §406 of the CWA (BAV)

Regulations (HAWAII)

- Each state develops recreational water quality STANDARDS based on EPA RWQC
- HAWAII recreational water quality standards

(HAR Chapter 11-54-8) (2014):

Based on Enterococci:

- GM 35 CFU/100 ml (over 30 day)
- STV 130 CFU/100 ml (10% of samples within 30 day period)
- Hawaii does NOT close beaches due to the indicator bacteria levels, we do post caution and warning signs

Regulations

EPA Recreational Water Quality Criteria (RWQC) (2012)



Hawaii recreational water quality information:

Hawaii Department of Health Clean Water Branch http://health.hawaii.gov/cwb/



Challenge I:

Small sample volume issue

- Current monitoring programs evaluate water quality based on 100 ml sample volumes while overwhelming evidence shows that <u>microbial (including indicator</u> <u>bacteria) concentrations vary vastly in space and time</u>
- <u>Sample volumes used in current monitoring programs are often not sufficient</u> for the detection of many source specific markers and human pathogens

Huge concern in coastal environments as such a small water sample is not representative of the actual water quality.

Analyses of large sample volumes is needed.





Hawaii's legendary Waikiki beach deserted after sewage spill



An employee of the Hawaii Department of Health takes a water quality sample at Ala Moana Beach Park in Honolulu.



Ultrafiltration (UF)

Wide array of options:

- Design (tubular, spiral, plate, etc)
- Tangential-flow and dead-end options
- Pore size
- Material
- etc.







DEUF based sample concentrator - PMACS

(Portable Multi-use Automated Concentration System)



- Dead-end ultrafiltration device
- Automated
- Hollow fiber filter cartridges
- 150 kDa
- >100 L
- Light and portable

Recovery module

Collection module



| E A | | | | ΓΡΑΕΙΙ ΤΡΑΤΙΟ | NI (100L) | | | GRAB SAM | MDIF (11) | |
|---------------------------------------|--|--------------|-------------------|------------------|-----------------------------|--|--|------------------|-------------------------|-------------------|
| | | ~~_ N ⊢ | 011 | Enterococci /M | /IPNI/100ml) | | | | | 1/100ml |
| Piwai | | Site | 6/13/2017 | 6/20/2017 | 6/27/2017 | 7/5/2017 | 6/13/2017 | 6/20/2017 | 6/27/2017 | 7/5/2017 |
| Reservoir | | ζ Λ 1 | <0.04 | 13.02 | 0.08 | 0.04 | < 10 | < 10 | < 10 | <10 |
| A A ASSOCIATION | | 2 | >97 | 0.432 | 4.892 | 24.52 | 441 | < 10 | 10 | 20 |
| | | 3 | 0.04 | 0.54 | 1.196 | <0.04 | < 10 | < 10 | < 10 | <10 |
| A A A A A A A A A A A A A A A A A A A | kauai | 5 4 | >97 | 83.6 | 168.8 | 226 | 1145 | 160 | 292 | 364 |
| | | 5 | >97 | 8 | 29.2 | 153.2 | 1565 | 109 | 109 | 235 |
| SITE | 5 | | | C. perfringens | (CFU/100ml) | | | C. perfringens | (CFU/100ml) | |
| uali Hight < | | Site | 6/13/2017 | 6/20/2017 | 6/27/2017 | 7/5/2017 | 6/13/2017 | 6/20/2017 | 6/27/2017 | 7/5/2017 |
| Kan | ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~ | 1 | <0.04 | 0.2 | 0.08 | 0.16 | < 10 | < 10 | <10 | <10 |
| | | 2 | 6.8 | 1.52 | 6 | TM? | 10 | < 10 | 30 | 10 |
| | | 3 | <0.04 | <0.04 | 0.12 | <0.04 | < 10 | < 10 | <10 | <10 |
| | | 4 | 32.8 | TM | 108 | 56 | 70 | 120 | 70 | 290 |
| 11 | | 5 | 3.12 | TM | 180 | 32 | 140 | 120 | 140 | 40 |
| | | | | F+ coliphage (| PFU/100ml) | | | F+ coliphages | (PFU/100ml) | |
| | | Site | 6/13/2017 | 6/20/2017 | 6/27/2017 | 7/5/2017 | 6/13/2017 | 6/20/2017 | 6/27/2017 | 7/5/2017 |
| 1 22 | | | <0.04 | <0.04 | <0.04 | <0.04 | < 10 | <10 | <10 | <10 |
| | SITE 4 SITE 1 | | <0.04 | <0.04 | <0.04 | <0.04 | < 10 | <10 | <10 | <10 |
| \mathbf{X} | | 3 | <0.04 | <0.04 | <0.04 | <0.04 | < 10 | <10 | <10 | <10 |
| | | 4 | 1.68 | <0.04 | 0.16 | 0.24 | 20 | <10 | <10 | <10 |
| | | | <0.04 | <0.04 | <0.04 | <0.04 | | | <10 700 (DELL/100ml) | <10 |
| | t- Black and a second | Site | 5 6/13/2017 | 6/20/2017 | e (PFU/100111) 6/27/2017 | 7/5/2017 | 6/12/2017 | 6/20/2017 | 6/27/2017 | 7/5/2017 |
| | | | <0.04 | <0.04 | <0.04 | <0.04 | < 10 | <10 | <10 | <10 |
| Y ` 🔪 🖉 | | 2 | 1.6 | <0.04 | 0.08 | 0.28 | 10 | <10 | <10 | <10 |
| | | 3 | <0.04 | < 0.04 | < 0.04 | < 0.04 | < 10 | <10 | <10 | <10 |
| | | 4 | 32 | 8 | 21.6 | 37.44 | 140 | 30 | 80 | 240 |
| | | 5 | TM | 5.2 | 14.4 | 14.64 | 450 | 120 | 70 | 70 |
| | | | Human-asso | ciated Bacteroid | les (HF183/287 |)(gc/100ml) | Human-assoc | ciated Bacteroid | des (HF183/287) | (gc/100ml) |
| | Poipu R. | Site | 6/13/2017 | 6/20/2017 | 6/27/2017 | 7/5/2017 | 6/13/2017 | 6/20/2017 | 6/27/2017 | 7/5/2017 |
| | | 1 | negative | negative | negative | negative | negative | negative | negative | negative |
| Poipu Beach | Rd | 2 | 10426 | negative | 1679 | 2703 | negative | negative | negative | negative |
| SITE 3 | IERATON | 3 | 380 | negative | negative | negative | negative | negative | negative | negative |
| | | | 4021 | negative | 38196 | negative | <loq< th=""><th>negative</th><th>negative</th><th>negative</th></loq<> | negative | negative | negative |
| SITE 2 | | 5 | negative | negative | negative | negative | negative | negative | negative | negative |
| | | | Рерр | er mild mottle | viruses (gc/100 | ml) | Pepp | er mild mottle | viruses (gc/100) | ml) |
| | | Site | 6/13/2017 | 6/20/2017 | 6/27/2017 | 7/5/2017 | 6/13/2017 | 6/20/2017 | 6/27/2017 | 7/5/2017 |
| | | | negative | negative | negative | negative | negative | negative | negative | negative |
| | | | negative | negative | negative | negative | negative | negative | negative | negative |
| | | | negative | negative | negative | 305 | negative | 312 norotiur | negative | negative |
| | | DEACH PARK 4 | negative AEQOQ | 555 702717 | 100482 | 10000000000000000000000000000000000000 | negative | 10/01/ | negative | negative 33080 |
| | | | 43608 | 233/4/ | 100463 | 204211 | 27233 | 104914 | 0100 | 22203 |
| | | SILEI | | | | | | | | |

SITE 5

SITE 4

Hanakaape Bay

<u>100 m</u>

Pros:

Provides more **<u>representative</u>** sample

Excellent for detection of rare targets (such as pathogens)

Can pick up 28 nm sized viral particles (150 kDa cutoff)

Utilizes disposable filters which are reasonably priced (\$30) (avoids cross-contamination)

Cons:

Labor and time intensive

Further concentration steps needed for molecular work (~370 ml)

PCR inhibitors can be a challenge

Cleanup-contamination concern

Choose wisely!



Challenge II:

Current methods slow, need for rapid beach notifications

Sewage spill now estimated at 500,000 gallons; Visitors urged to stay out of Waikiki water 2015 Posted: Aug 24, 2015 11:07 AM HST

Updated: Aug 24, 2015 7:06 PM HST PHOTOS: Severe Weather hits Hawaii

START SHARE 58/60



THE BEAT

2013 Sewage Spills Into Ala Wai Canal

A broken sewage pipe in Makiki has leaked 4,400 gallons of wastewater into a culvert leading into the Ala ...

OCTOBER 19, 2013 · By Civil Beat

f Share 🛛 🕑 Tweet 🖪 Share

THE BEAT

Keoki Kerr Leaves TV For HSTA 💭 0 31 MINUTES AGO - By Chad Blair State Population Up 5.2

Percent In 5 Years MARCH 24, 2016 - By Bianca Smallwood Despite Indictment, Kenoi Won't Resign

MARCH 24, 2016 - By Anita Hofschneider

Mayor Wright Redevelopment Q2 Advances MARCH 24, 2016 · By Anita Hofschneider

Hawaii Superferry To Run In Q7 Canada MARCH 24, 2016 - By Nathan Eagle

LATEST STORIES

A broken sewage pipe in Makiki has leaked 4,400 gallons of wastewater into a culvert leading into the Ala Wai Canal.

The state Department of Health has posted signs warning residents to stay out of the canal in the area of South King Street and Kalakaua Ave.



NEWS 2015 Hawaii's legendary Waikiki beach deserted after sewage spill

By Associated Press

August 26, 2015 | 9:56am



An employee of the Hawaii Department of Health takes a water guality sample at Ala Moana Beach Park in Honolulu



ACAUTION

Wastewater bacteria

cause illness

may be in river and ma

Call State Department of Health 808-586-4309 for information

WEATHER V WAKE UP 2DAY V LIVING808 V SPORTS V TRAFFIC V REPORT IT NEWS V

2014

Honolulu Harbor off-limits after wastewater spill, runoff pollutes Oahu's south shore

KEEP OUT OF WATE Manolo Morales and Brigette Namata ublished: October 20, 2014, 8:58 am | Updated: October 20, 2014, 6:24 pm

💟 🕒 😭 🕖

Honolulu Harbor remains off-limits after wastewater spill 20 MILLION GALLONS OF SPILLED SEWAGE



CAUTION

Call State Department of Health at 808-586-4309 for information



KEEP OUT OF WATER

WASTEWATER BACTERIA WERE DETECTED YESTERDAY AND YOU SHOULD NOT HAVE SWUM

WATER QUALITY MIGHT BE OK TODAY

PLEASE CHECK OUR WEBSITE TOMORROW

Based on a slide from Dr. J. Griffith presentation



qPCR (cce per 100 mL). All subjects, marine and fresh water beaches combined.



^a Enterococci measured using EPA Method 1600 (U.S. EPA, 2002a), or another equivalent method that measures culturable enterococci.

^b *E. coli* measured using EPA Method 1603 (U.S. EPA, 2002b), or any other equivalent method that measures culturable *E. coli*.

^c EPA Enterococcus spp. Method 1611 for qPCR (U.S. EPA, 2012b). See section 5.2.



Method 1611: Enterococci in Water by TaqMan[®] Quantitative Polymerase Chain Reaction (qPCR) Assay Method 1609: Enterococci in Water by TaqMan[®] Quantitative Polymerase Chain Reaction (qPCR) with Internal Amplification Control (IAC) Assay

Environmental Protection

Agency

October 2012

March 2013

Hawaii and rapid methods

HI extremely well suited:

- ~8 million tourists per year, many high use beaches (Waikiki beaches, Ala Moana)
- High use beaches are easy to reach, hence easy to sample and post

Rapid accurate methods would make difference (ruining on not ruining a person vacation)



Water is good to swim today



http://www.sccwrp.org/



Same day response

Improved service to visitors and residents



• ...and growth of enterococci in extra-enteric environments

ADDITIONAL PARALLEL TEST(S) NEEDED!

Challenge III:

High background levels of indicator bacteria in HI: Any from sewage? (microbial source tracking)





Figure EO-2: Sources of Pollution That Caused Closings/Advisories, 2000-2011

Total days shown are greater than annual totals because more than one pollution source may have contributed to each closing/ advisory. **Key:** (A) Sewage spills and overflows. (B) Polluted runoff, stormwater, or preemptive due to rain. (C) Unknown. (D) Other reasons (including closings/advisories with no source information provided).

NRDC, 2012: "Testing the Waters"

No science to close the beach

High background levels of indicator bacteria in HI: microbial source tracking

The STANDARD - enterococcus (facultative anaerobe):

- is not human specific
- grows in Hawaii soils, and other extra-enteric environments
 - Luther & Fujioka (2004): 3.2 56,000 CFU/g, (n=5)
 - Kirs (unpublished): <1 >2,420 MPN/g, GM=46 (n=33)

The sewage tracer – C. perfringens (obligate anaerobe)

- is not human specific
- remains viable for long periods in sediments



Microbial Source Tracking (MST):

Set of tools to identify the source of fecal contamination in ground and surface waters

US: 10⁹ tons/year

Agricultural and Human Sources of Feces in the U.S.



Importance:

- Different Health Risk!
- Management!
- Mediating Conflicts!

HUMANS

Human Noroviruses, Human Adenoviruses

RUMINANTS E.coli O157:H7 Cryptosporidium Giardia

BIRDS

Salmonella

Campylobacter

Cultivation or microscopy based techniques aren't able to distinguish between source specific strains...



...but molecular techniques can.





Target organisms

- Bacteroidales
- Methanobrevibacter
- F⁺ specific RNA Coliphages
- Different groups of viruses
- Enterococci
- E. coli
- and others ...



Strategy

- site survey
- local knowledge and historical data
- case by case basis
- multi-tiered approach
- single sample has little or no value (wet/dry)
- single marker has little or no value

Important:

1. Markers need to be validated on local population for specificity (false positives) and sensitivity (prevalence in host population)



2. Ideally die-off (decay) of the markers in given region water matrix should also be identified

3. Ideally verify ability to discriminate between fecal sources by blind testing mixed samples:



How to determine sewage source: Manoa watershed study



Nine sites analyzed monthly for one year for concentrations of:

- 1) enterococci, E.coli, C. perfringens,
- 2) two sewage markers (HF183, HPyV), and
- 3) bacterial community structure







N ≜

| | In violation | | | | | | | | |
|---|--------------|--------|--------|--------|--------|-------|--------|--------|-------|
| | MS-1 | MS-2 | MS-3 | MS-4 | MS-5 | MS-6 | MS-7 | MS-8 | MS-9 |
| Enterococcus stand. exceeded | 9 | 12 | 12 | 12 | 12 | 12 | 5 | 4 | 1 |
| (%) | (75) | (100) | (100) | (100) | (100) | (100) | (41.7) | (33.3) | (8.3) |
| C. perfringens STV exceeded | 0 | 4 | 7 | 8 | 9 | 6 | 1 | 1 | 0 |
| (%) | (0) | (33.3) | (58.3) | (66.7) | (75.0) | (50) | (8.3) | (8.3) | (0) |
| In violations with the Hawaii | 0 | 4 | 7 | 8 | 9 | 6 | 1 | 1 | 0 |
| standard and STV (both exceeded) (%) | (0) | (33.3) | (58.3) | (66.7) | (75.0) | (50) | (8.3) | (8.3) | (0) |



| Human markers | | | | | | | | | | |
|---------------|-------|--------|-------|-------|-------|-------|-------|-------|-------|--|
| Site: | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | |
| Human- | | | | | | | | | | |
| associated | 2 | 13 | 12 | 11 | 12 | 11 | 9 | 10 | 5 | |
| Bacteroides | (15%) | (100%) | (92%) | (85%) | (92%) | (85%) | (69%) | (77%) | (38%) | |
| Human | 0 | 5 | 8 | 8 | 7 | 6 | 0 | 1 | 1 | |
| Polyomavirus | (0%) | (38%) | (62%) | (62%) | (54%) | (46%) | (0%) | (8%) | (8%) | |



| Site | # of | Number | Shannon | Simpson | Fisher (α) | Evenness (J) |
|------|---------|---------|---------|---------|------------|--------------|
| | samples | of OTUs | | | | |
| WWTP | 10 | 79 | 1.51 | 0.63 | 5.30 | 0.40 |
| MS-1 | 2 | 377 | 4.16 | 0.95 | 51.27 | 0.77 |
| MS-2 | 3 | 449 | 3.71 | 0.90 | 42.78 | 0.70 |
| MS-3 | 3 | 456 | 3.76 | 0.93 | 42.16 | 0.71 |
| MS-4 | 3 | 279 | 2.51 | 0.76 | 22.65 | 0.54 |
| MS-5 | 3 | 348 | 2.88 | 0.82 | 29.78 | 0.58 |
| MS-6 | 3 | 408 | 3.58 | 0.92 | 32.51 | 0.71 |
| MS-7 | 3 | 138 | 2.60 | 0.82 | 10.51 | 0.61 |
| MS-8 | 2 | 113 | 3.00 | 0.89 | 11.88 | 0.69 |
| MS-9 | 3 | 167 | 2.43 | 0.83 | 11.32 | 0.56 |



Sewage related fingerprint was detected at all sites form MS-2 to MS-5

Manoa watershed: conclusions

- Sewage related impairment by three lines of evidence: indicator bacteria, MST markers, and bacterial community structure comparisons
- Massive sewage leak: probably not
- Markers support enterococci <u>AND</u> C. perfringens based monitoring
- Ala Wai not so bad (dilution)
- Old sewer lines and illegal cross-connections/hookups universal problem



Take Home

- Enterococci grow in Hawaiian soils, hence do not necessarily reflect health risk associated with sewage borne contaminants. Therefore posting beaches solely based on the concentrations of enterococci can: 1) harm public opinion about Hawaii, and 2) decrease public attention to beach postings
- Analyzes of larger sample volumes provides better representation of water quality and can be used to detect microbes which are present at low concentrations such as many pathogens. Yet you need to be smart when to use ultrafiltration.
- Rapid molecular methods can provide beach water quality information a few hours after sample collection and are well suited for popular beaches in Hawaii.
- Several options exists to detect sewage in water samples

