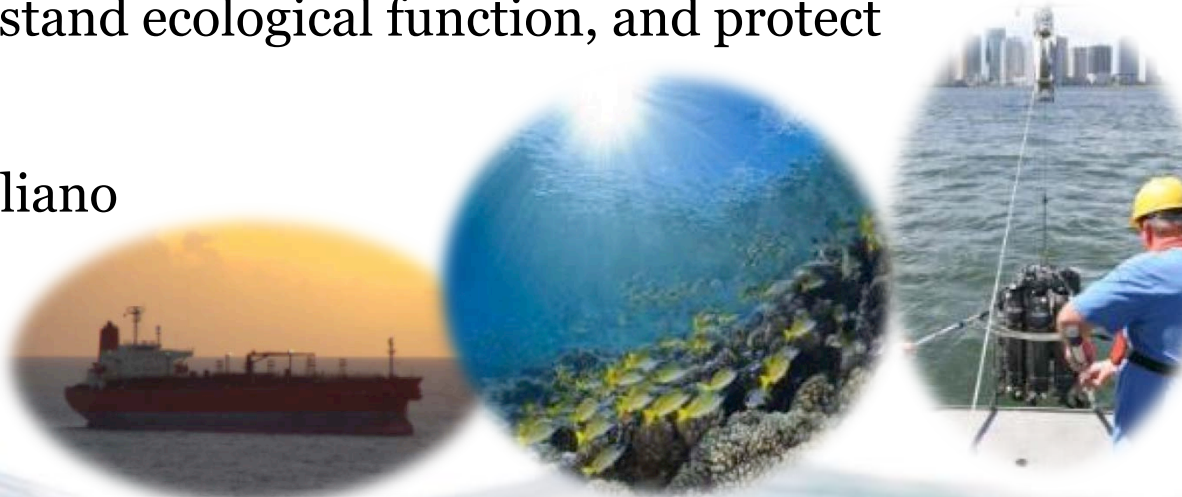


# Oceans and Ecosystems Research

## **Molecular & Environmental Microbiology**

to explore biodiversity, understand ecological function, and protect health

Kelly Goodwin & Chris Sinigalliano

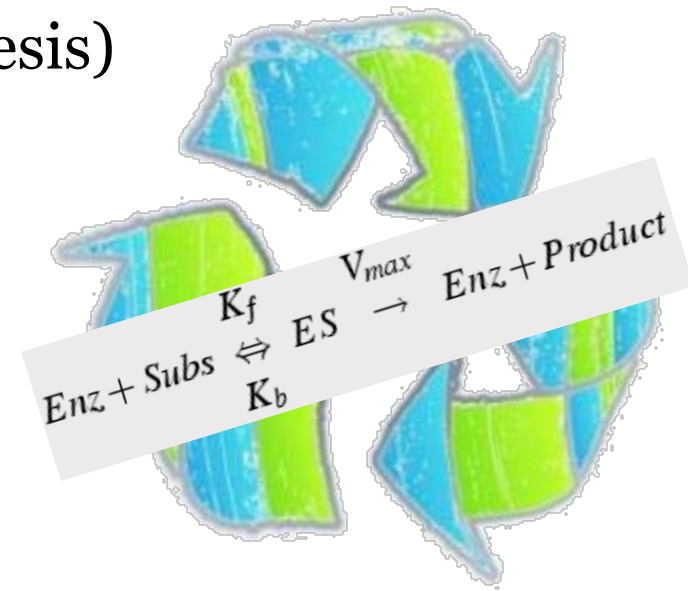


# Microbial Dynamics Underpin Ecosystem Function

*(they control...pretty much everything)*

## **Good:**

- Nutrient Cycles (including photosynthesis)
- Climate-controlling gases
- Ozone-depleting gases
- Waste Treatment
- Degrade toxic compounds
- Life-saving medicines



## **Bad:**

- Impair Water Quality (including oxygen depletion)
- Diseases & Infections of fish, protected species, corals, humans

# *Main Science Questions*

- What are the sources of degraded water quality in coastal ecosystems\*?
- How can we improve microbial water quality assessments (faster, easier, more accurate) and transfer that knowledge to stakeholders?
- How can we best harness the power of 'omic technologies to characterize a main driver of the Earth System – microbial diversity & function.

## *For what purpose?*

- Better protect health, ecosystems, and economies from sewage pollution, pathogens, and harmful algae.
- Improve understanding of ecosystem diversity and function to improve assessment and stewardship of ecosystem services



\*geographically specified system of organisms (including humans), the environment, and the processes that control its dynamics. –  
*NOAA Five Year Research and Development Plan 2013-2017*



# Microbial Water Quality Assessments

Applications include recreational water & seafood safety, regulatory compliance, diseases of protected species & commercial fish

## Recreational Water Focus – Beaches Are Bucks:

- ✓ **85% of US tourism dollars** are from coastal recreation with the creation of **1.6 million jobs** in 2000. CA beaches: **>100 million visitors** annual. FL beaches: **\$19.1 billion** from out-of-state tourists in 2003.

## Repercussions of degraded water quality:

- Microbial contamination makes people sick.
- Contamination (or fear of) adversely affects consumer confidence.
- Local governments forced to spend millions \$\$ annually on monitoring.
- Mitigation is mandated - costs can be 100's of millions \$\$\$\$.



- **Links to NOAA Strategic Plan**
  - Holistic Understanding of the Earth System through Research
    - Multiple links, including specific Implementation Plan Inclusion (transformative research & innovation/Research to Operations)
  - Engagement Enterprise
  - Resilient Coastal Communities and Economies
    - Improved coastal water quality supporting human health and coastal ecosystem services
  - Healthy Oceans
    - Improved understanding of ecosystems to inform resource management decisions; Recovered and healthy marine and coastal species
- Other NOAA Links: Microbe and Ecosystem Health Working Group, One Health Working Group, Ecoforecasting Pathogen Team, OAR Ecosystem Portfolio, 2013 National Ocean Policy Implementation Plan

# Partnerships and Stakeholders

Public Health Agencies, Fisheries, Tourism Industry, Environmental/Conservation Organizations, Concerned Citizens, Beach Managers, Water Quality Managers, Academic Institutions, Environmental and Public Health Scientists



SPYGLASS™



Southern California Coastal Water Research Project



AOML Program Review

# AOML Molecular and Environmental Microbiology



- Development, Validation, and Transfer of Molecular Tools
- Application of Microbial Water Quality Assessments for:
  - coral reef and coastal ecosystem health research
  - investigation of ocean and human/animal health interactions
- ‘Omic tools to characterize Earth System drivers:
  - microbial diversity and function controlling, e.g., global biogeochemistry, food webs, biological response to change
- Research Mentorship and International Engagement



Why are molecular tools needed for microbial water quality assessments?



# Traditional Methods are too Slow

Actually, these are yesterday's results. Check back tomorrow to see if the surf was ok today.

“NRDC Sues EPA for Failing to Update Obsolete Water Quality Standards” - 2006



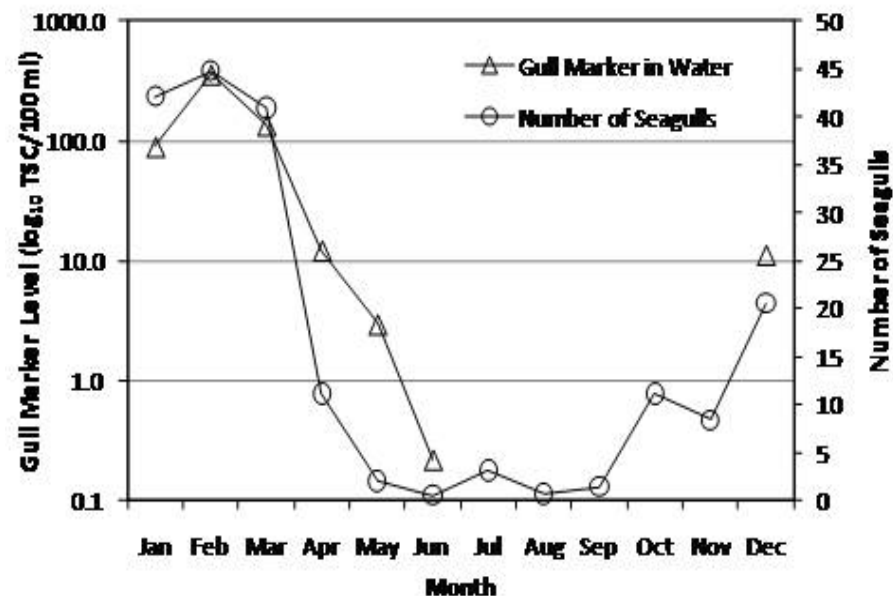
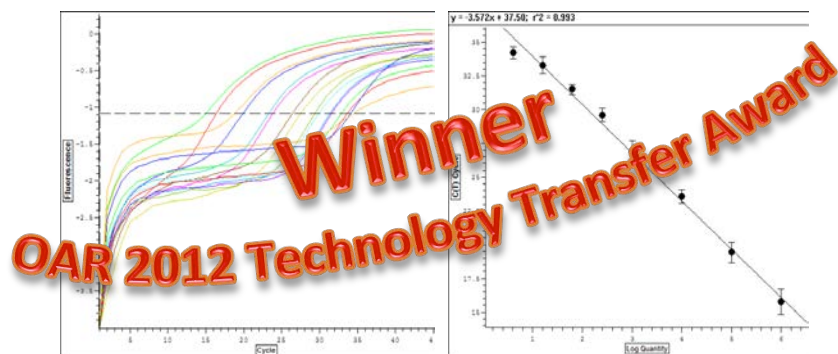
Consent decree lead to new EPA guidelines (2012) and spurred research on molecular and automated methods

# Microbial Source Tracking (MST)

MST identifies contamination sources (e.g., humans, birds, dogs) to decrease risks to health and to preserve coastal economies. MST helps managers devise mitigation strategies and assess whether investments are working.

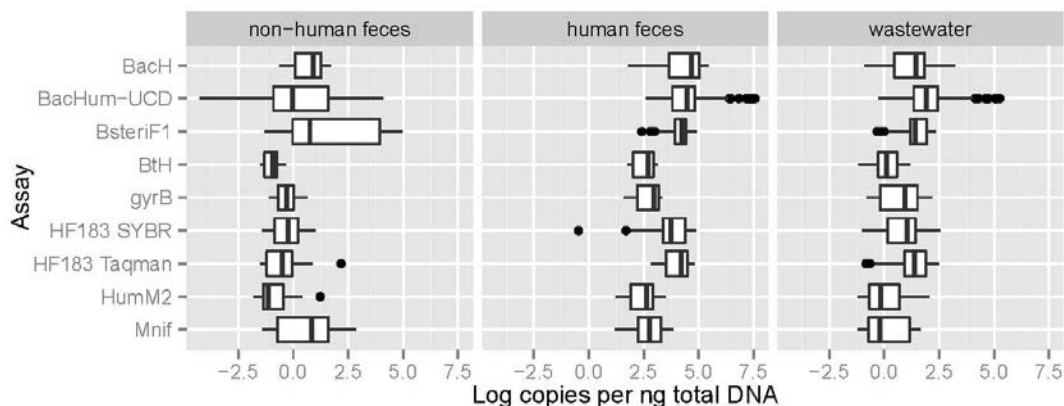
## AOML MST assays for dog and gull contamination:

- validated -- large state-funded project
- transferred -- academia, industry, state & local governments. Protocols provided in the 2013 "California Microbial Source Identification Manual"



AOML gull assay matched the number of gulls measured by automated cameras

# AOML inter-laboratory validation efforts contribute to state and national guidelines



Examples:

- EPA National Exposure Research Laboratory, Multi-Laboratory Validation Study for enterococci and Bacteroidales qPCR.
- EPA Office of Water, Inter-Laboratory Validation Study of EPA fecal indicator qPCR assays in support of revised 2012 recreational water quality criteria.
- State of California, Source Identification Protocol Project (SIPP) for 41 MST methods tested by 27 laboratories.

Assay	Sensitivity			Specificity		
	Human <i>n</i> <sup>a</sup>	DNQ+	DNQ-	Non-human <i>n</i> <sup>a</sup>	DNQ+	DNQ-
BacH	12	100%	75%	26	77%	85%
BacHum-UCD	72	97%	97%	156	37%	67%
BsteriF1	48	100%	96%	104	44%	61%
BtH	12	100%	92%	26	54%	96%
gyrB	12	92%	50%	26	58%	96%
HF183 endpoint	84	75%	NA	182	96%	NA
HF183 SYBR	48	100%	92%	104	78%	89%
HF183 Taqman	60	100%	95%	130	46%	92%
HumM2	72	93%	67%	156	75%	94%
Mnif	60	78%	60%	130	68%	76%

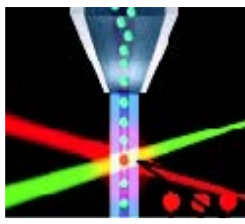
AOML testing of multiple human-associated fecal markers for the SIPP study. HF183 Taqman emerged as one of the recommended assays for human MST.

# Development, Validation and Transfer of Molecular Tools

Since 2000, AOML has developed and evaluated a variety of platforms for molecular assays. Goals: faster, cheaper, more accurate, high throughput, multiple target detection



colorimetric microplate



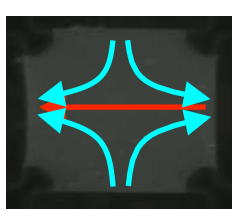
suspension array



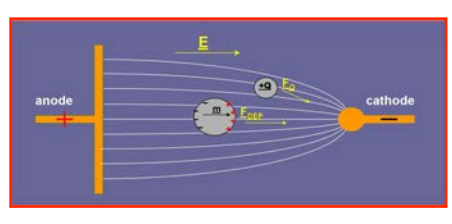
electrochemical



QPCR

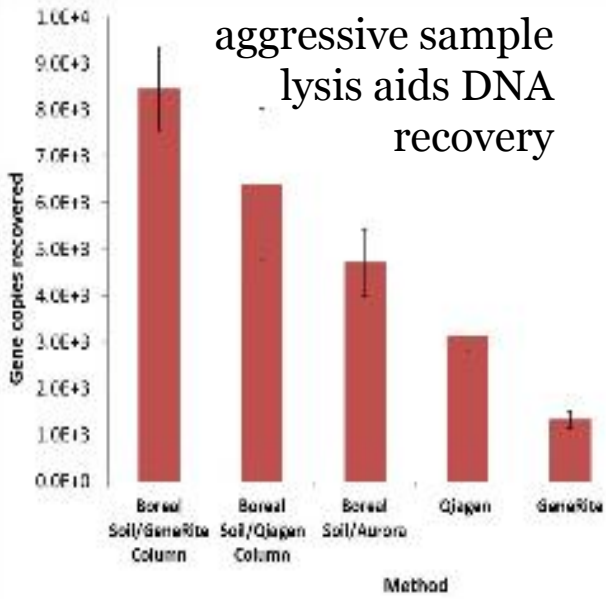


SCODA

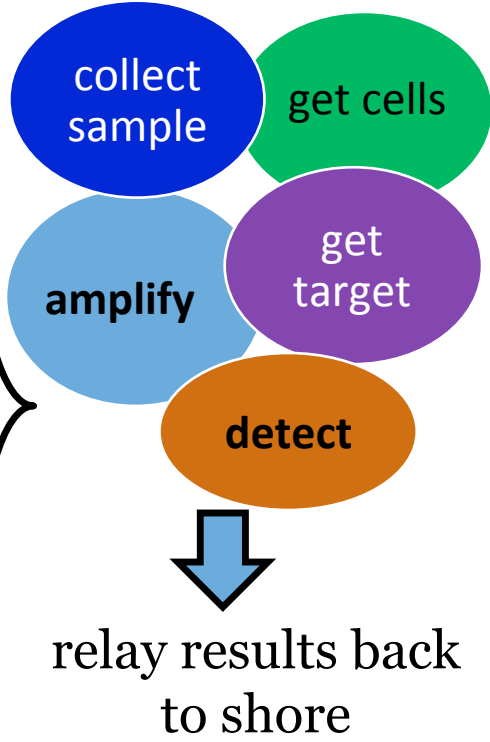


whole cell dielectrophoresis

Recent work includes sample preparation methods for in situ, automated biosensing platforms, such as the Environmental Sample Processor (ESP)



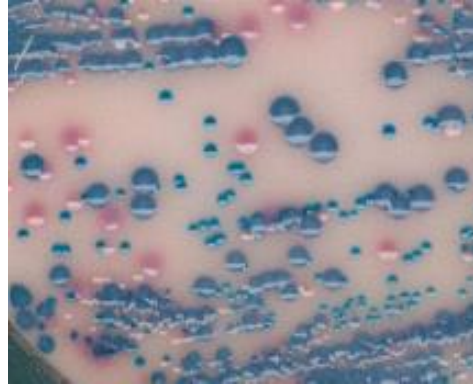
<http://www.mbari.org/ESP/default.htm>



# Application of Microbial Water Quality Assessments



Land-Based Pollution



Epidemiology



Protected Species



Natural Source  
Exclusion



BMP Evaluation

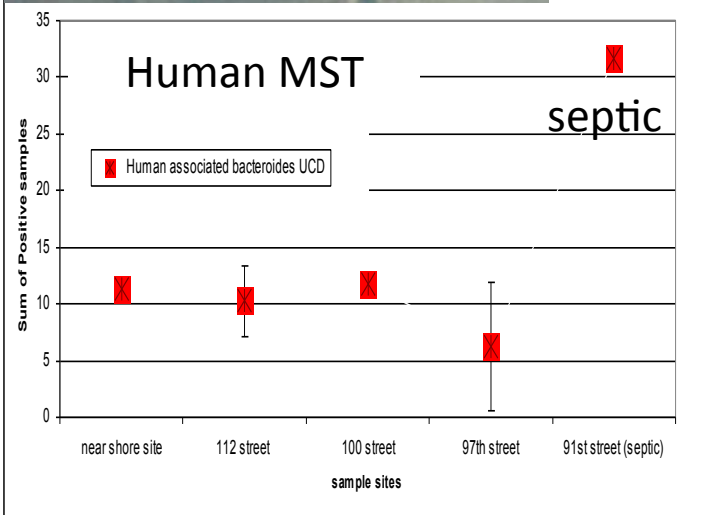


Coral Pathogens

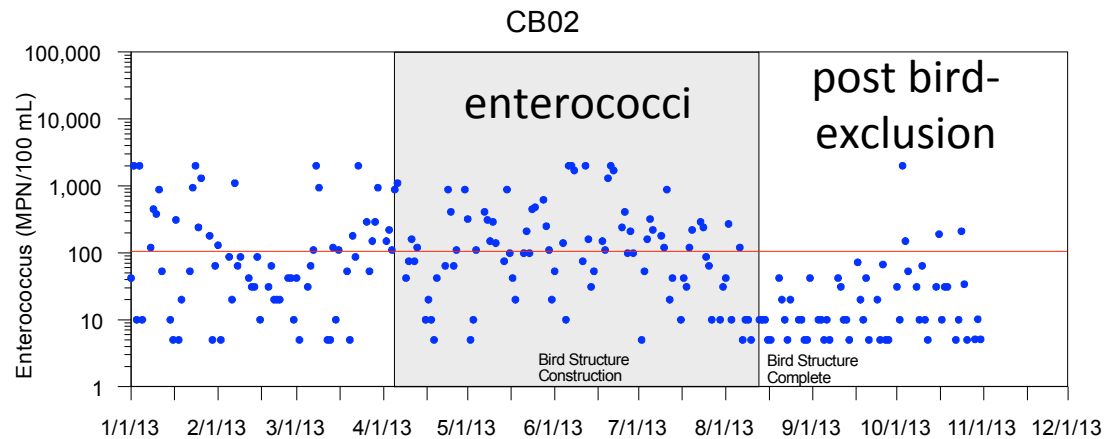
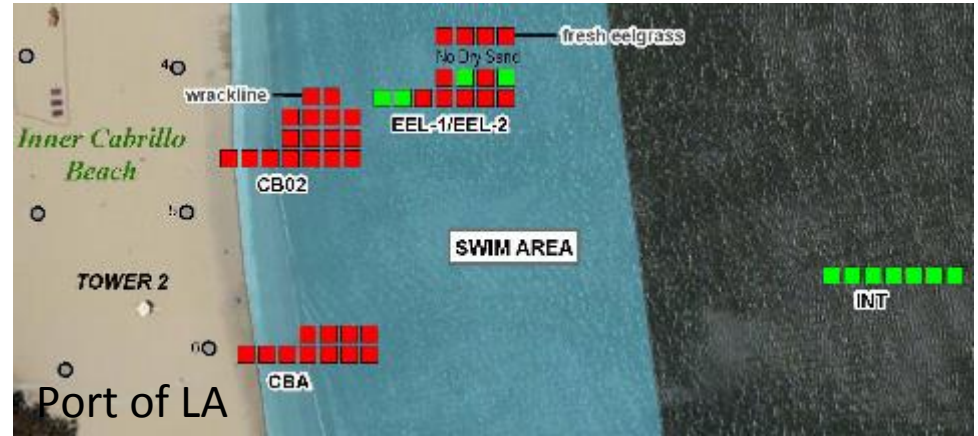
## Example Studies:

- FL Keys National Marine Sanctuary Little Venice Canal
- F.A.C.E. Program
- Tijuana Watershed
- Hobie Beach Baseline
- High Intensity Diurnal
- Epidemiology – FL (B.E.A.C.H.E.S)
- Epidemiology – CA (CA Water Study)
- Bather Shedding
- Coastal MSSA & MRSA
- Sand Pathogens
- Pathogen/Indicator Environmental Persistence
- DOH/DOE Event Response
- Zoonotic Pathogens
- Seaweed Wrack & Sand
- Poche Beach Watershed
- Cabrillo Beach TMDL
- Coral Pathogen, *S. marcescens*

# AOML MST efforts guide and evaluate remediation efforts



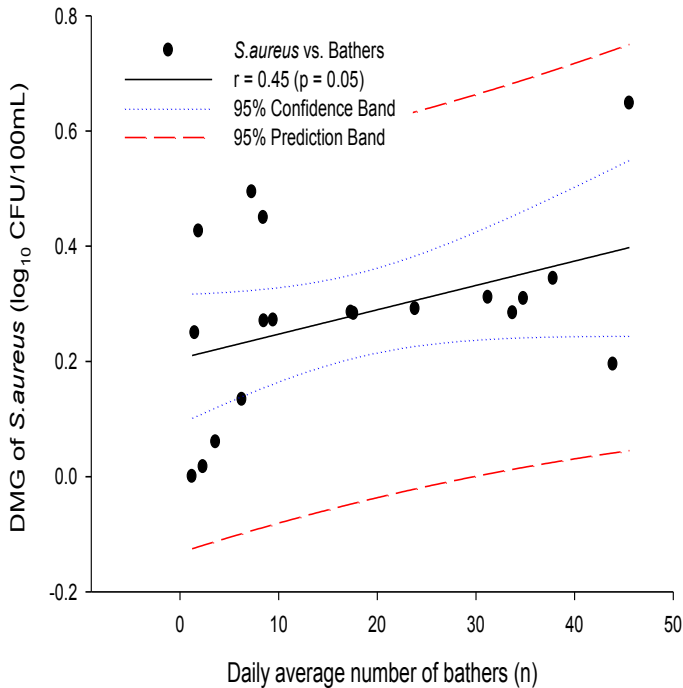
AOML testing of residential canals in the Florida Keys indicate that switching from septic to sewer improves water quality.



AOML MST assay shows widespread contamination from gulls. Fecal indicator bacteria concentrations drop after completion of a bird-exclusion structure.

# Application of Microbial Water Quality Assessments: for investigation of ocean and human/animal health interactions

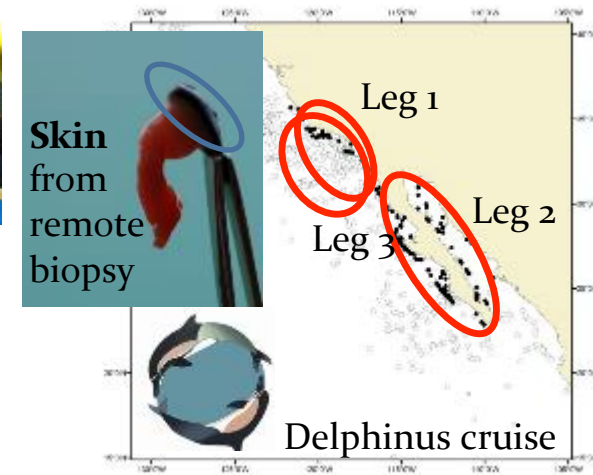
## Pathogen detection is used to characterize mechanisms of contamination and risk assessment for humans & animals



*Staphylococcus aureus* (MSSA and MRSA) in seawater correlated with the number of human bathers, suggesting that humans are a source of beach contamination.



Pathogen research leads to recommendations for human-animal interactions during rehabilitation.



Pathogen load on dolphin skin tracks to distance of habitat from shore, suggesting impacts from land-based sources of pollution.

$$SA (\ln \text{ CFU/L}) = -4.6 + 0.58 * SA (\ln \text{ CFU/100 dryg}) + 0.22 * \text{watertemp}(C) + 0.22 \text{ ENT} (\ln \text{ CFU/L}) + 0.018 x \text{ swimmers}$$

Multivariate linear regression describes *S. aureus* concentrations.

# The 'omic revolution: transforming medical, food, energy, and environmental sciences



<1%



Metagenomics & microbes: circumvents the obstacle of laboratory cultivation

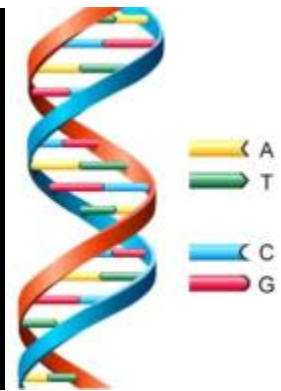
from this



to this

The pace of technology:  
100 DNA sequences to 100 Million  
DNA sequences per run in 10 years

```
AGGGGCGGCAGCTTACACATGCA
AGTCGAGCGGTAGCACAAGGAGA
GCTTGCTCTCTGGGTGACGAGCGG
CGGACGGGTGAGTAATGTCTGGG
AAACTGCCTGATGGAGGGGGATA
ACTACTGGAAACGGTAGCTAATA
CGCATAACGTCGCAAGACCAAAG
AGGGGGACCTTCGGGCCTCTTG
```



Transformational Research:

*“...Revolutionizing entire disciplines, creating entirely new fields, or disrupting accepted theories and perspectives.”*

-National Science Foundation

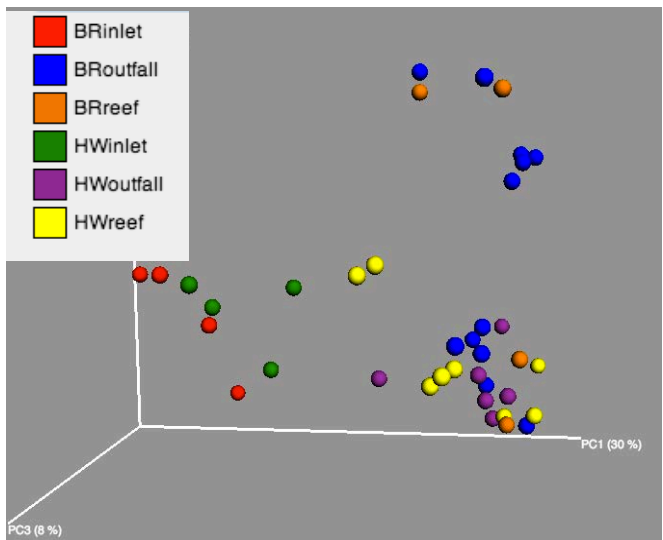


‘Omic tools to characterize Earth System drivers:

# Metagenomics to uncover microbial diversity & function, set ecosystem baselines, and support ocean exploration



Samples collected pre-DWH showed metabolic pathways consistent with the ecosystem response to the spill. Methanotrophic pathway coverage was similar in shallow (200m) and deep (650m) samples; BTEX degradation and methanogenesis coverage was enhanced in deep samples.



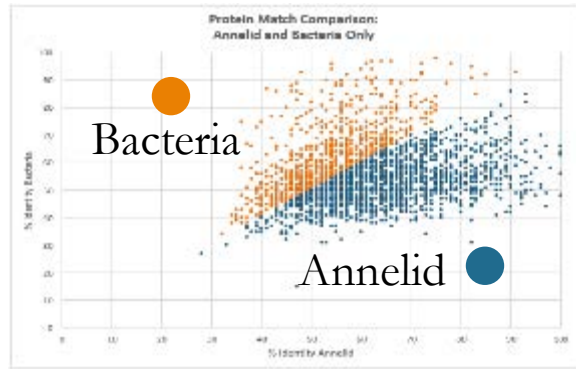
Analysis of next-gen sequencing reveals differences in microbial communities for outfall, inlet, and coral reef seawater samples.



Methane hydrate covered in methane ice worms

Bacterial Order	%	Archaeal Order	%
Campylobacterales	27	Methanosarcinales	55
Alteromonadales	12	Methanomicrobiales	18
Desulfobacterales	8	Methanobacteriales	12

Gut samples from the rare methane ice worm -- dominated by organic, sulfur, and methane degraders.



Bioinformatic extraction of methane ice worm genome from a mix of bacterial and archaeal sequences.



## Science Education and Research Mentorship at NOAA AOML: Providing Opportunities for the Next Generation

Examples:

- NOAA Hollings Scholars
- NOAA-Smith College Program Interns
- Environmental Protection Agency Interns
- University Research Assistants
- NSF Research Experience for Undergraduates
- High School Research Interns



**Captivating**

**Interdisciplinary**

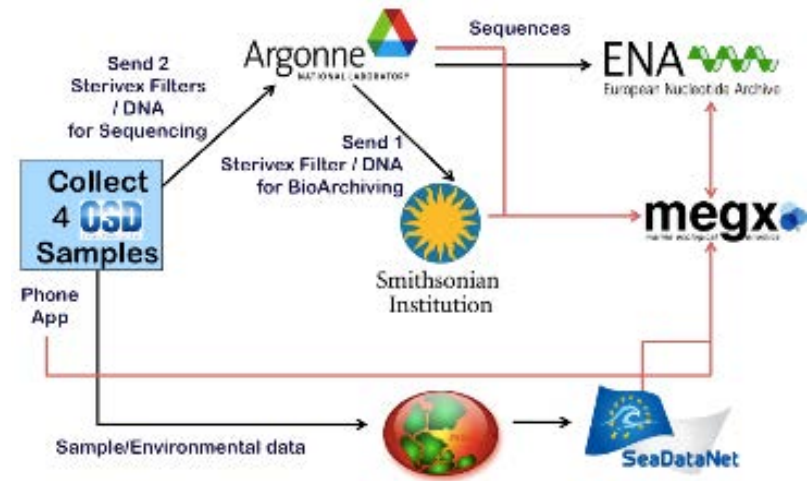
**Cutting-edge**



## EU-US Commission Joint Consultative Group

### 2013 Meeting Opportunities for Collaboration in Marine Microbial Ecology

- **Short-term tangible identified:** NOAA participation in Ocean Sampling Day (OSD), a worldwide synchronized sampling of seawater for metagenomic analysis using internationally-accepted protocols for sampling, shipping, analyzing, storing and data archiving. **Summer Solstice June 21-22, 2014.**



- **New funding:** Establish Coral Reef Genomic Observatories to characterize microbial community structure, biodiversity, and land-based pollution source impacts in Gulfstream Reef tracts by next-generation sequencing & community genomic characterization.

# Questions?

