



Molecular Technologies and Managing for a Sustainable Environment: Opportunities And Challenges

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OUR ENVIRONMENT

- Much of our economy and quality of life is dependent on healthy ecosystems
 - Productive agriculture and forestry
 - Clean air & water
 - Human health and well-being
- We make large investments in maintenance and monitoring to ensure healthy environment
- Molecular methods are presently only a tiny fraction of that investment
 - NIST currently has only a minor role in environmental quality monitoring and assessment

MANY OPPORTUNITIES FOR INCORPORATING NEW TECHNOLOGIES

- Assessing the environment
- Deriving value from the environment
- Cleaning the environment
- Some of the greatest opportunities occur through development of novel biological discoveries – many motivated by the genomics revolution



CHALLENGES IN MEETING THESE OPPORTUNITIES

- Who validates the new methods?
- How do we interpret new data in context of old standards?
- Can we effectively transfer these technologies to the commercial sector and the user community?
- What is a standard in the life sciences?
 - Is there a biological equivalent to weights and measures?
 - What is NIST's unique role in addressing these challenges?



ASSESSING THE ENVIRONMENT

- Human health monitoring
 - Fecal contamination on swimming beaches
 - Harmful algal blooms
 - Seafood safety and quality
- Environmental health monitoring
 - Barcoding to assess presence of natural biological communities
 - Microarrays to measure sublethal effects
 - ELISA to quantify chemical exposure
- Species identification for ecosystem management
 - Detection of invasive species in ballast water
 - Identification of distinct fishery stocks
 - Endangered species monitoring in foods, such as fish roe and fillets

BEACH WATER MONITORING



Beachgoers feel protected

The Problem



Results in 24-96 hrs



Reality

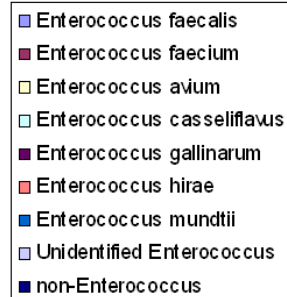
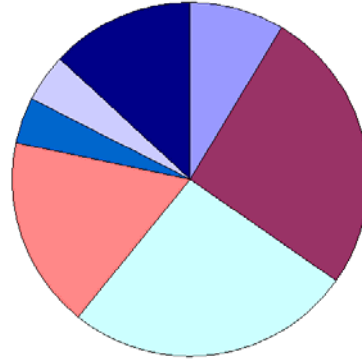
The Solution



Results in 2 hrs

IT'S NOT JUST ABOUT SPEED

- Accuracy
 - Culture based methods are non-specific
 - This leads to false positives
- Host Specificity
 - We currently target indicators that grow well in culture
 - Other less-cultivable organisms are more specific to the human intestinal tract
 - More false positives!

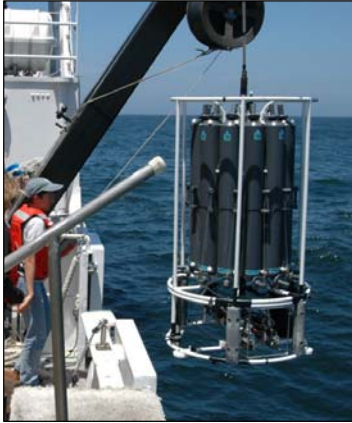


HARMFUL ALGAL BLOOMS

- Several dozen algal species release toxins
 - Bird and mammal effects
 - Shellfish bed closures
- Other algae cause discoloration and odors
- Economic impacts
 - Lost shellfish harvesting
 - Respiratory illness
 - Recreation and tourism

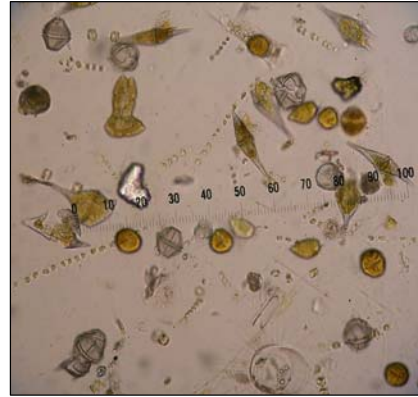
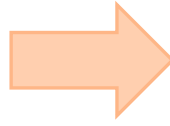


CURRENT MONITORING FOR HARMFUL ALGAL BLOOMS



Ship-based water collection

- Labor-intensive
- Results can take weeks



Place sample on slide



Manually count species

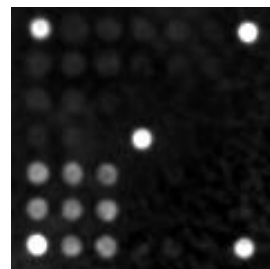
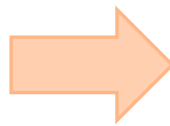


FUTURE DETECTION OF HARMFUL ALGAL BLOOMS



Moored Pumping Array

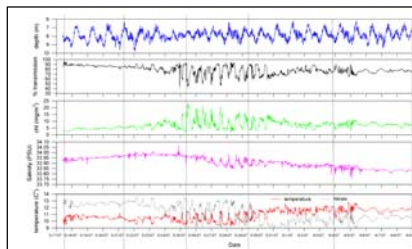
Continuous Data Available on the Web



In-Situ Microarray Detection



Real Time Telemetry of Data to Shore



Automated Assimilation into Nowcast and Forecast Models



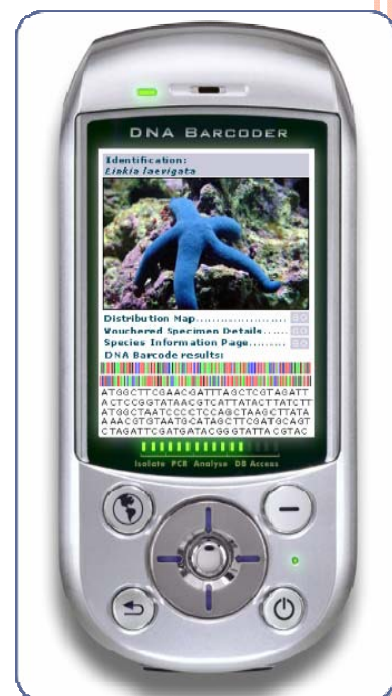
NATURAL BIOLOGICAL COMMUNITIES

- “Balanced Indigenous Species” is a foundation of the Clean Water Act
 - Biology is a monitoring program focal point
- Existing methods are slow
 - Samples need to be hand sorted and identified
 - Requires specialized taxonomists who are in short supply
 - Often takes months



BARCODING AS AN ALTERNATIVE

- Build a reference library
 - Gather tissue samples from well-identified specimens
 - Extract, amplify and sequence DNA
 - Data submission to GenBank
- Use the reference library
 - Collect and sequence unidentified specimens
 - Compare with reference sequences
- Many advantages
 - \$10 vs. \$1,000
 - Hours vs. months for data availability



WE ALREADY HAVE BARCODES FOR 50,000 SPECIES

BOLDSYSTEMS Management & Analysis

Hesperidae of the ACG 1 (CSCR)

Specimen Identifiers

Sample ID: 02-SRNP-16276 Mission ID: 02-SRNP-16276
 Isolate - Field Name: Collection Code: [Edit Specimen](#)
 Donated By: Deposited In: Smithsonian Institution

Taxonomy

Identifier: 19
 phylum: Arthropoda
 class: Insecta
 order: Lepidoptera
 family: Hesperidae
 subfamily: Pyrginae
 genus: Anastus
 species: Anastus obscurus

Specimen Details

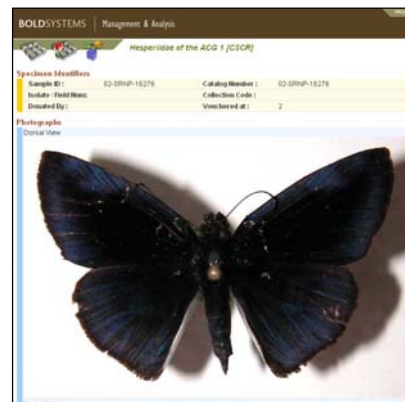
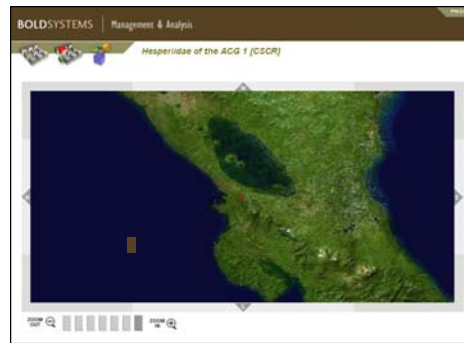
Voucher Type:
 Tissue Type:
 Extra Info: Pyrginae
 Sex: m
 Reproduction: s
 Life Stage:

Collection Data

Collectors: Rosler Moraga
 Date Collected: 12-Jul-2002
 Country: Costa Rica
 State/Province: Ouanacoste
 Region/County: Area de Conservacion 276
 Sector: Del Oro
 Exact Site: Urcaria
 Latitude: 11.0291
 Longitude: -85.4792
 Coord. Source:
 Elevation/Depth: 300

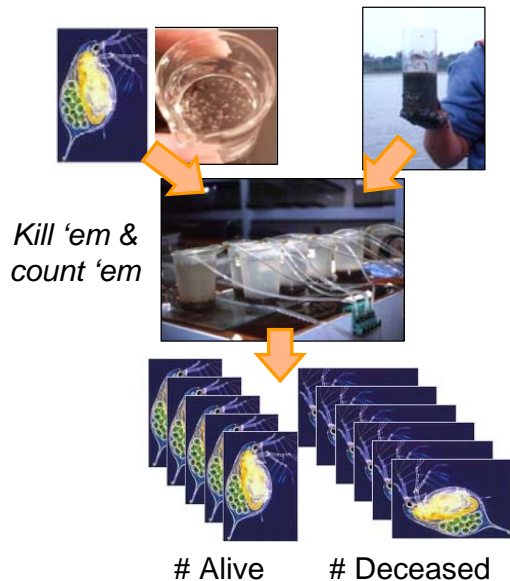
Photographs

Dorsal View Ventral View



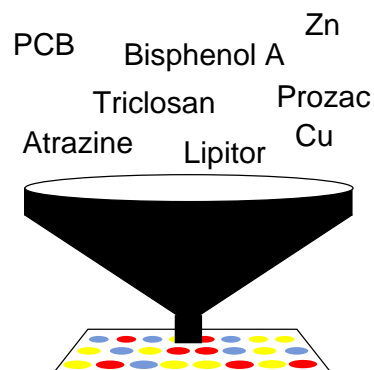
SUBLETHAL EFFECTS

Traditional Toxicity Tests



- Doesn't address sublethal effects well
- Which chemicals are responsible?

Molecular Methods



- Microarrays test for specific chemicals or mixtures
- Low cost enzyme assays assess sublethal effects across species

INVASIVE SPECIES

- Introduced species often proliferate because natural predators and competitors are absent
 - The economic consequences can be large



Zebra mussels clog a water intake pipe



Discharging ballast water

- Cargo ship ballast water is the most frequent vector for invasives
- Genetic screening can provide a rapid means for assessing the threat



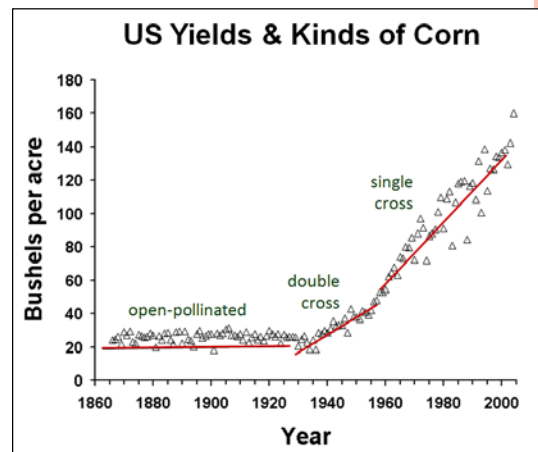
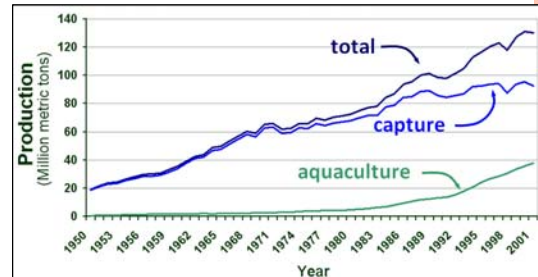
DERIVING ECONOMIC VALUE FROM THE ENVIRONMENT

- Aquaculture presently contributes 40 million tons of seafood
 - This will likely double within the next decade
- Algal-based biofuels are in development
 - The promise of energy independence
- Both use genomics to improve economic returns
 - Genetically modified organisms challenges of aquaculture and biofuels are similar to those with agriculture



AQUACULTURE

- Global demand for fishery products is increasing
 - Natural fish stocks are becoming depleted
 - Aquaculture will fill the gap
- Aquaculture is still in the early stages of productivity increases
 - Unclear whether that will be achieved through selective breeding or transgenic means
- Either way, contamination of natural genetic stock is of concern



SEAFOOD QUALITY

- Species authentication
 - Are you eating what you paid for?
 - Are protected species making their way to your dinner plate?
 - Another application for barcoding
- Proteomics to assess quality
 - Are we talking fish fry or sushi?
- Seafood safety
 - Are there parasites and pathogens present?
 - Rapid answers are needed?



CULTURED ALGAE AS A FUEL

The Allure

- Fastest growing plants on earth
- Some algae contain more than 50% oil
- They are a low-sulfur non-toxic fuel
- Global fuel requirements can be met using <5% of arable land



Open ponds in Hawaii

The Reality

- When they grow fast, algae make little oil
- When they make oil, they tend to grow slow
- Genetic engineering will probably be part of the equation



Closed Bioreactors

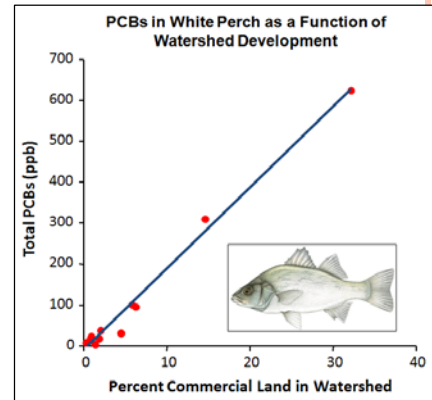
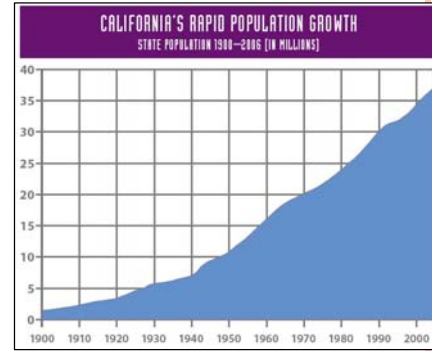
CLEANING THE ENVIRONMENT

- Traditional hazardous waste cleanup involves removal
 - Disrupts natural fauna
 - Contaminants can be hard to reach (e.g. wells)
 - Dredging releases contaminants into the water column
- *In situ* microbial decomposition (bioremediation) offers many advantages
 - Decomposition, rather than translocation, of wastes
- Genetic engineering can enhance success
 - *Deinococcus radiodurans* has been modified to digest toluene and mercury from radioactive nuclear waste
 - Molecular approaches provide unprecedented insights into key biodegradative pathways



INCREASING ECOSYSTEM PRESSURES

- Environmental issues are a growth industry
- Population growth will increase pressure on the environment
 - Food production
 - Energy supply
 - Contaminant loading
- There is a strong relationship between population density and contamination effects
- Climate change will further change the equation
 - Shifts in species distribution
 - Hydrological modification



CHALLENGES

- Method validation
 - Who determines that the methods achieve their desired goal?
 - What are the measurement standards?
- Data interpretation
 - These are new methodologies with little regulatory or historical context
 - Molecular methods measure on a different scale
- Technology transfer to the user community
 - Many technologies are at the leading edge, but the users are not
- Environmental threats (or perception of threat)
 - Spread of genetically modified organisms



GMOs: THE ELEPHANT IN THE ROOM



- Philosophical/religious concerns with gene manipulation
- Unforeseen effects from proliferation of transgenic organisms
- Legal definition of patent and property rights

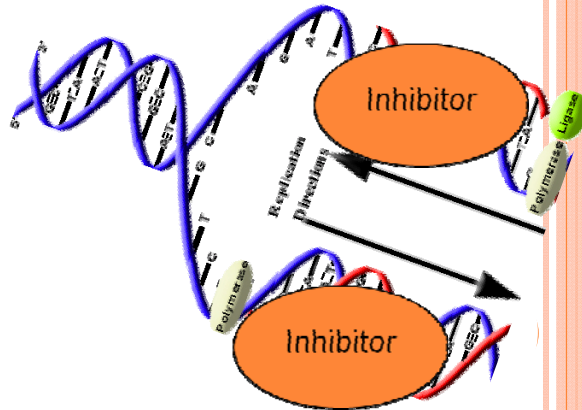
METHOD VALIDATION

- Are we measuring the correct things?
 - Have we identified the right sequence?
 - Is it consistent across geography?
 - Is it temporally stable?
- Are we measuring them correctly?
 - Are the methods repeatable across laboratories?
 - Is method sensitivity adequate?
 - Is there matrix interference?
- Unclear which organizations are responsible for making these determinations



INHIBITION

- Natural water samples contain compounds that can interfere with molecular assays
 - Humic acids
 - Polysaccharides
- Field tests of rapid beach bacteria methods found inhibition in 20% of samples
- Inhibition leads to underestimation
 - Health officials won't accept false negatives in a warning system
 - Need improved internal controls that detect inhibition



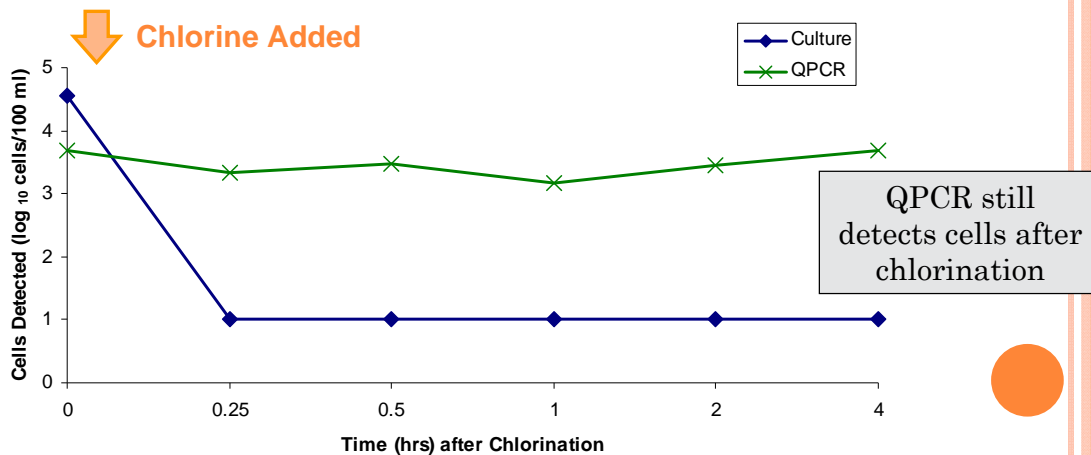
DATA INTERPRETATION

- We need thresholds for interpreting molecular data
 - Most methods don't distinguish live and dead material
 - What is sufficient evidence for proving a specimen belongs to a species? Who makes that decision?
 - Discovery is moving faster than linkage to legislation, regulation and litigation
- Quantitation
 - Many molecular approaches are presence/absence
- Trends assessments
 - Any methods replacement will compromise comparison to historic data



DETECTION OF DEAD BACTERIA

- Most molecular methods do not differentiate between viable and non-viable target organisms
 - Overestimates health risk
- Majority of wastewater outfalls are chlorinated



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TECHNOLOGY TRANSFER

- Many potential clients don't presently use molecular methods
 - Training needs will be extensive
- Methods need to be standardized
 - Many are still research methods
- Methods also need to be commercialized
 - Users can't typically obtain equipment and supplies from the research community
- Certification process to ensure that the local laboratories have been properly trained
 - Most states have laboratory certification process for traditional methods, but not for molecular methods



- **Mission ...to promote U.S innovation and industrial competitiveness by advancing measurement science, standards, and technology in ways that enhance economic security and improve our quality of life.**
- **Vision... become the world leader in creating critical measurement solutions and promoting equitable standards.**
 - We do this through:***
 - Standards Development and Deployment
 - Measurement Technology Development, Assessment, and Deployment
 - Measurement Science

POTENTIAL ROLES FOR NIST

- We need a national leader in methods evaluation
 - NIST has the appropriate mission and corporate history
 - State agencies are poorly equipped for this

- Methods standardization
 - Standard Reference Materials
 - Specimen banking
 - Data librarian?



NIST is already a leader in specimen banking

- Research to applications
 - Assist with commercialization
 - Training programs for local laboratories

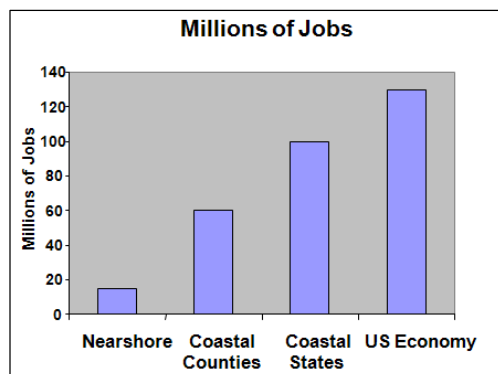
- All of these are similar to the NIST role in chemistry



COASTAL FOCUS

Coastal counties drive our economy

- Almost 50% of US jobs are located in coastal counties
- 55% of wages
- All on 7.6% of our land



Coastal economy depends on healthy biological systems

- Beach tourism
- Fishing
- Aquaculture
- Birdwatching / Whalewatching

