U.S. Coast Guard Aquatic Nuisance Species Program Overview

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U.S. Coast Guard
September 13, 2006
Prevention First 2006

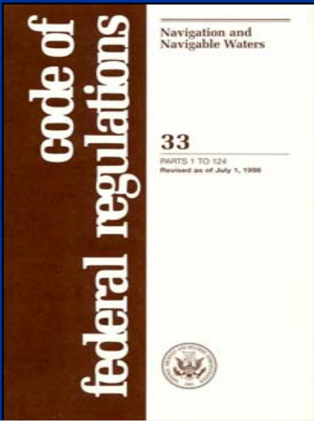
Ballast Water Laws & Regulations National

- Nat’l Invasive Species Act (NISA) 1996
  - Established National BWM program
  - Timeline – USCG Regulations (outside GL)

    1998 National Voluntary Program

    2001 Report to Congress: Voluntary program inadequate

    2004 Penalties and National Mandatory Program
  - 33 CFR 151 Subpart D



Discharge Standard Rule
In Progress

- **Environmental and regulatory analyses of alternatives**
  - **Scientifically sound, environmentally protective, enforceable**
- **Treatment system approval procedures**
  - **Notice w/Request for comments - August 2004**
  - **Conform to established USCG approval procedures**
  - **Informed by ETV, STEP, IMO, Others**

BWM Technologies

- Need to address full range of organisms including
  - Bacteria/microbes
  - All life stages
  - Those that reproduce asexually
  - Those not dependant on oxygen
- Need to be effective under wide range of conditions
- Single technology unlikely

BWM Technology Categories

- Mechanical
  - Filtration (screen, media*)
  - Separation (cyclonic, Ionic*)
  - Shear
- Chemical
  - Deoxygenation (pressure, N_2, inert gas generators)
  - Oxidizing biocides (hypochlorite, ClO_2, O3, AOT)
  - Non-oxidizing biocides (SeaKlean, gluteraldehyde*, acrolein)
- Energy
  - Radiation (UV, heat, ultrasonic)
  - Micro-Cavitation

Ballast Water Treatment Systems Biological Efficacy

- Do they work, generally?
- Difficult to answer at this point
  - No clear and comprehensive test reports available
    - System descriptions
    - Installation descriptions
    - Experimental design
    - Analytical methods
    - QA/QC
- Available data look promising with respect to IMO BW discharge standard
- No clear picture possible until implementation of standardized tests

Approving BWM Systems

- Type Approval of systems
  - Rigorous land-based testing
  - Shipboard qualification
- Consistent with existing USCG procedures and BWM Convention
- Requires development of standard test protocols
  - Efficacy in killing/removing wide range of organisms (bacteria – fish)
  - Under wide range of water quality conditions (blue water – turbid industrial estuaries)
  - Under wide range of operating conditions (temp, humidity, sea state)

EPA Environmental Technology Verification Program

Performance Verification of Ballast Water Treatment Technologies

ETV

EPA

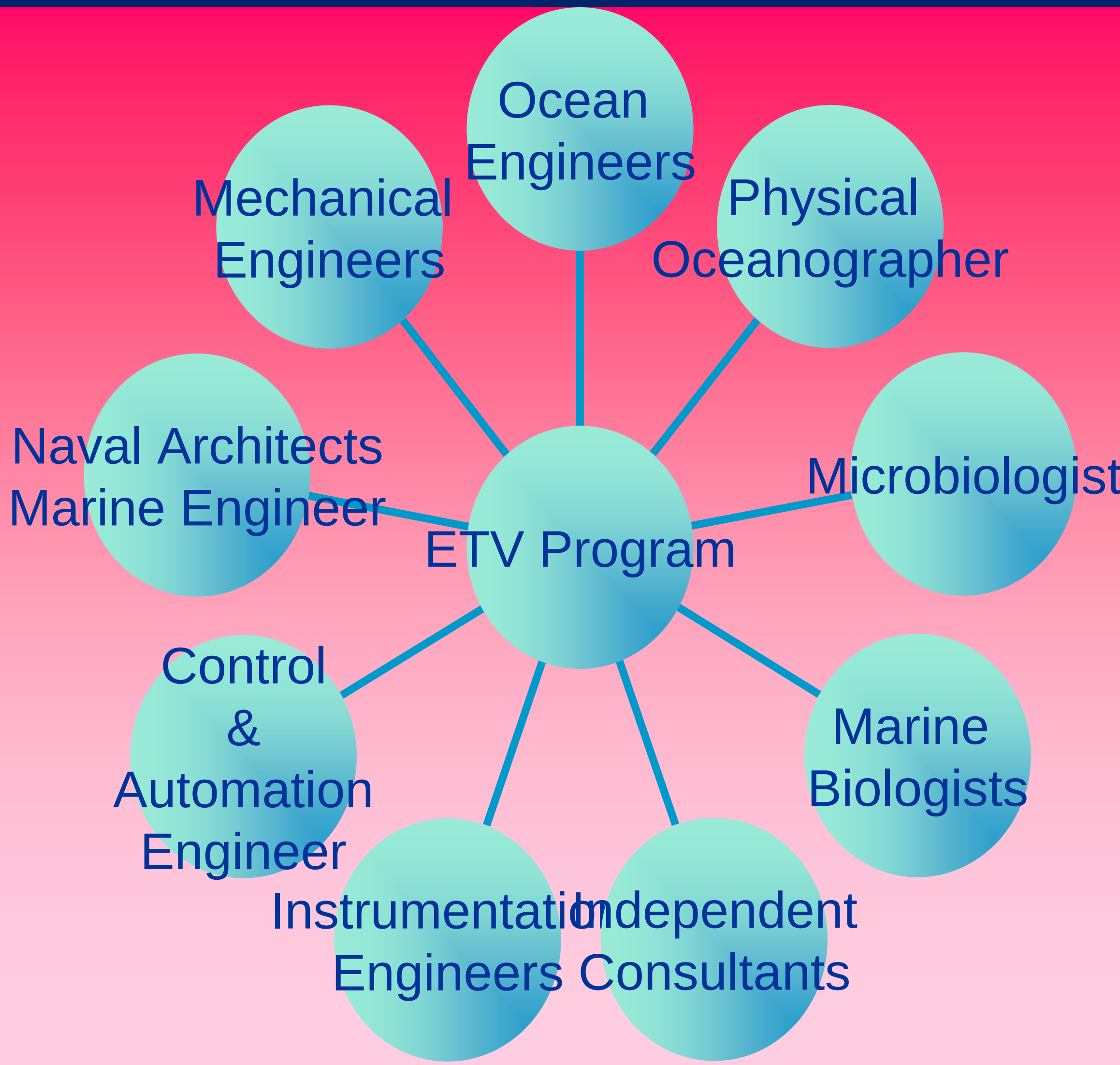
United States Coast Guard

NSF International

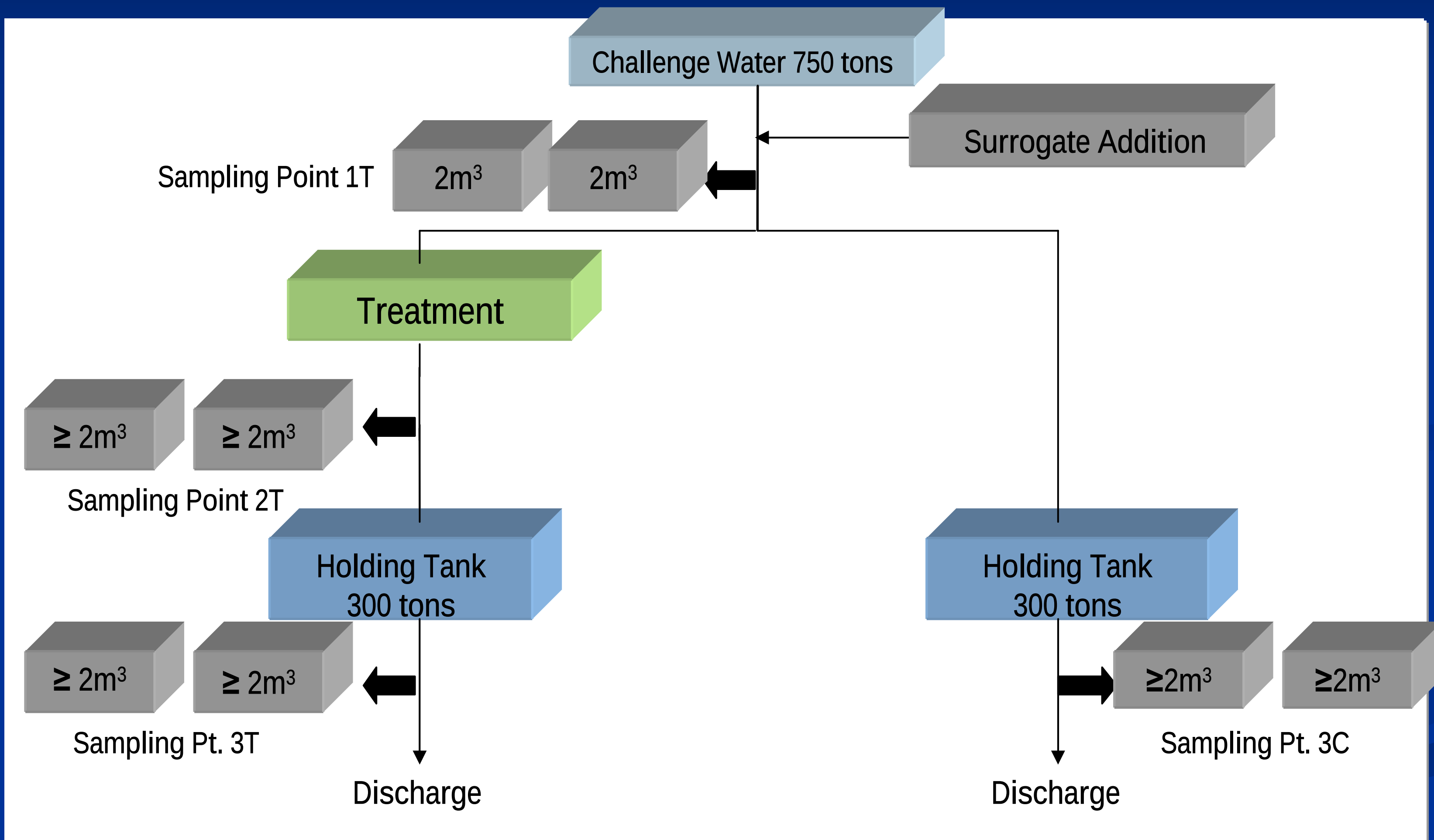
Battelle

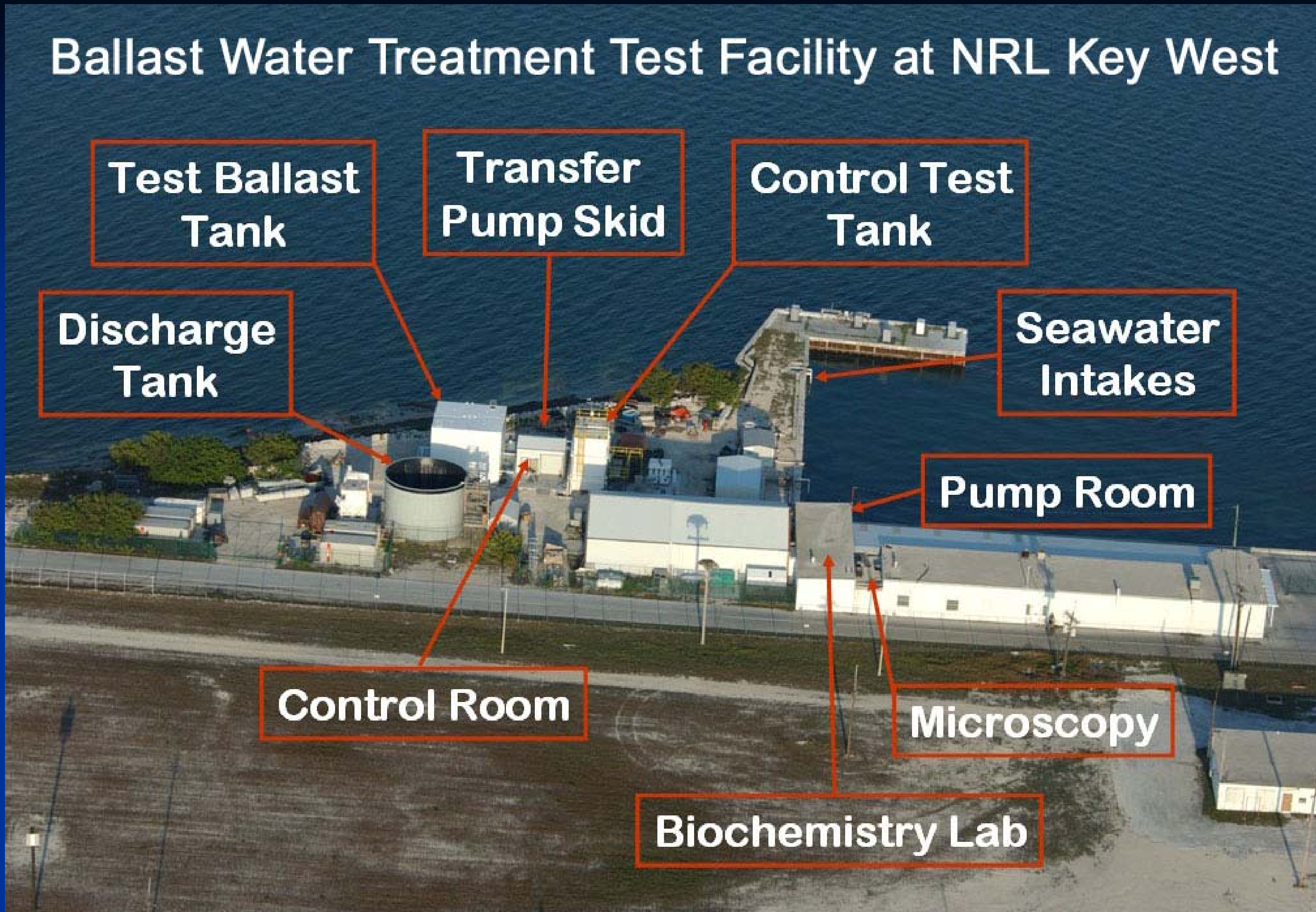
Coastal Resource and Environmental Management

ETV Tech Panel



Simplified Sampling Arrangements In-Line Treatment

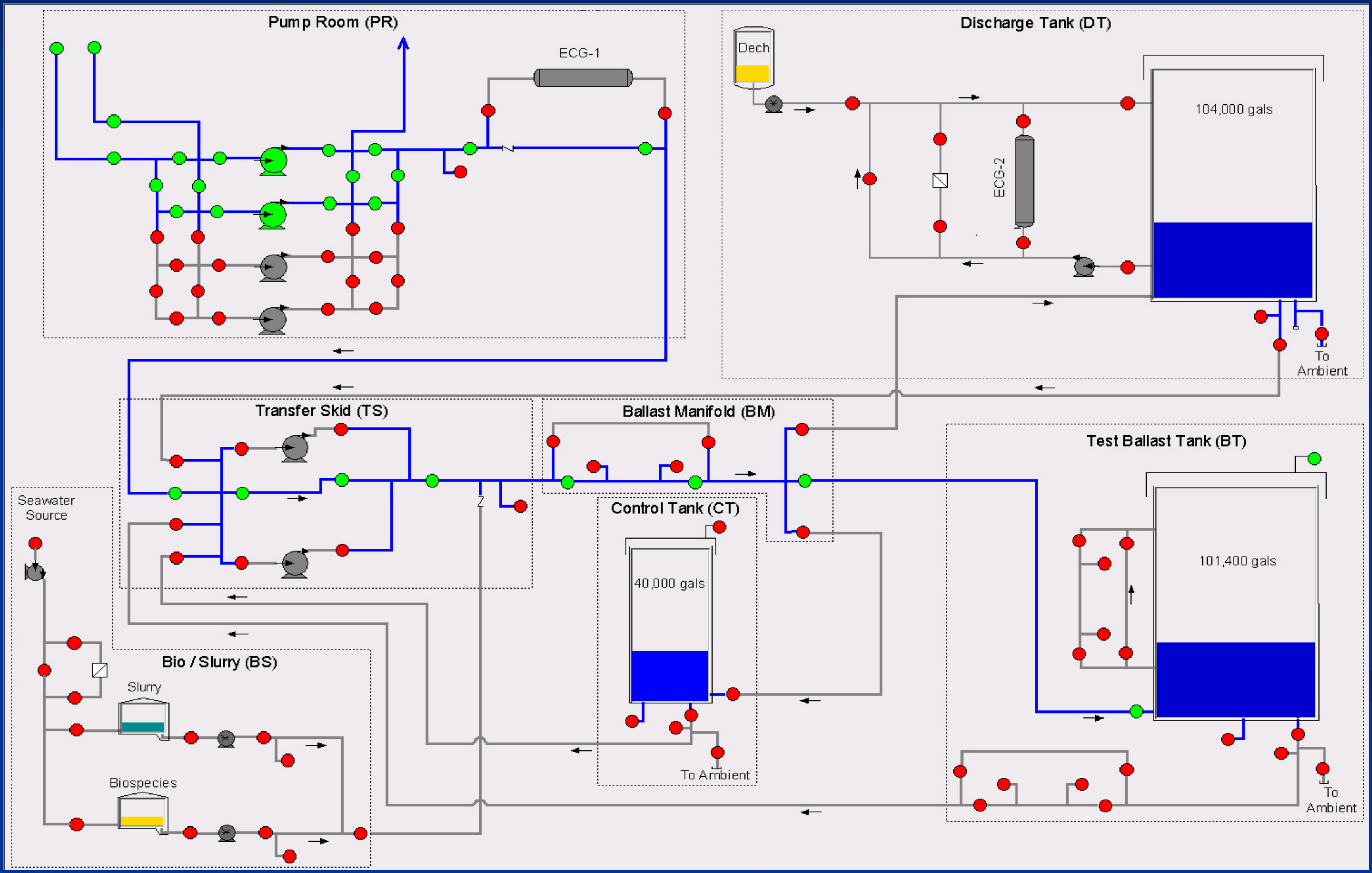




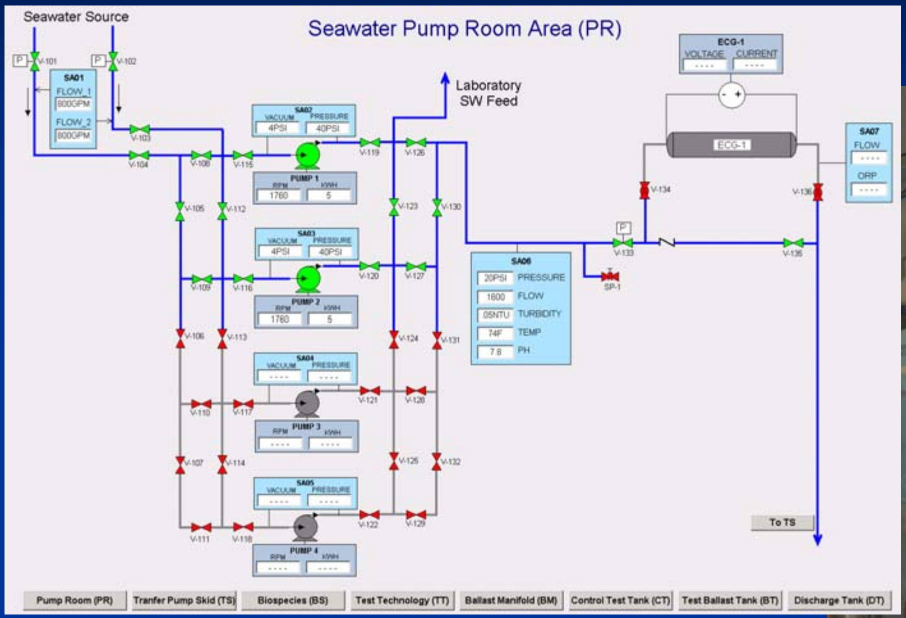
Center for Corrosion Science & Engineering
Naval Research Laboratory
Key West, FL

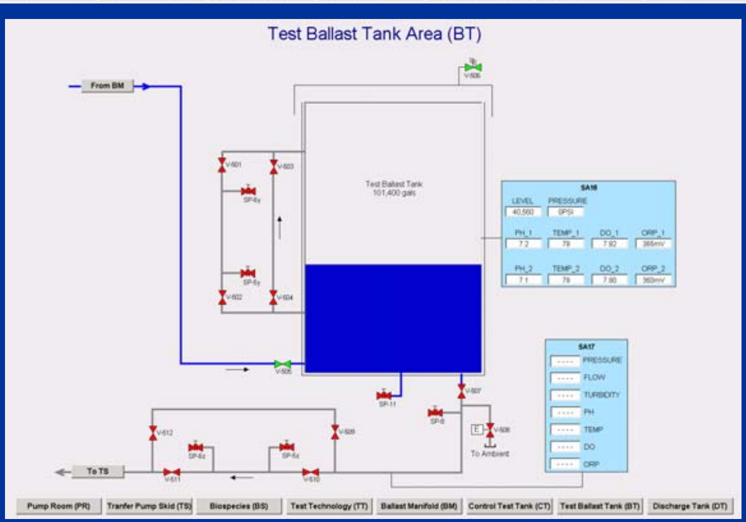
Test Facility Components

Automation, Control & Instrumentation



Instrumentation





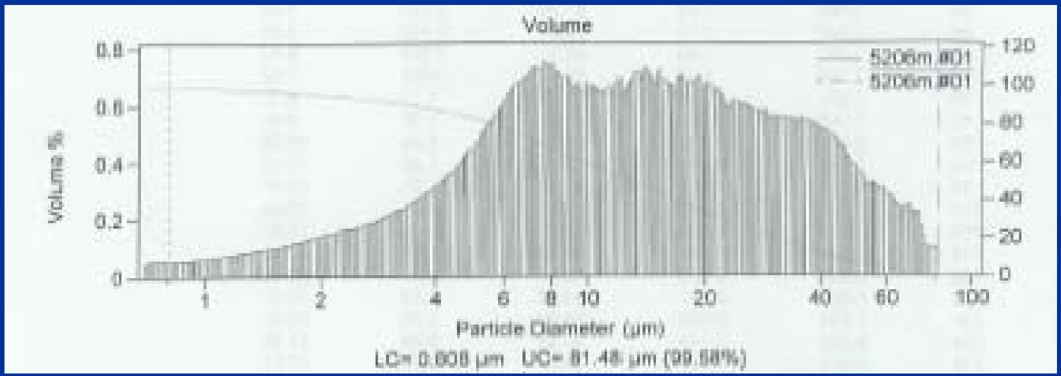
Instrumentation

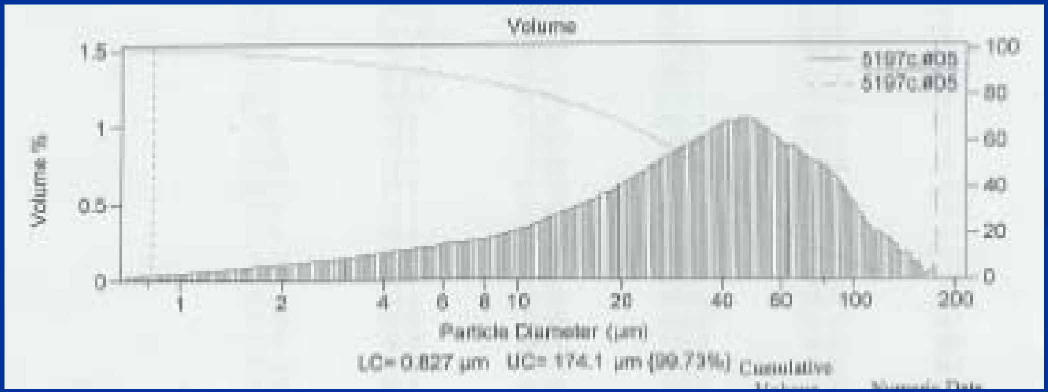
- Over 100 instrumented sensors for monitoring physiochemical properties at various locations.
  - Flow rate
  - Pressure & Differential Pressure
  - Temperature
  - Dissolved Oxygen
  - Turbidity (NTU)
  - pH
  - Particle Counts & Distribution
- 96 manual, pneumatic and electrically actuated valves:
  - Flow rate control
  - Isolation & Flow distribution
  - Sample acquisition
  - Each manual valve is wired with a magneto-sensor for open/closed information and supervisory monitoring (QA/QC purposes)
- Sample acquisition ports pre-BWT, post-BWT, post-tank
  - Organisms
  - Chlorophyll, POC, DOC, CHNP

Biological Laboratory

Methods: Particle Counting

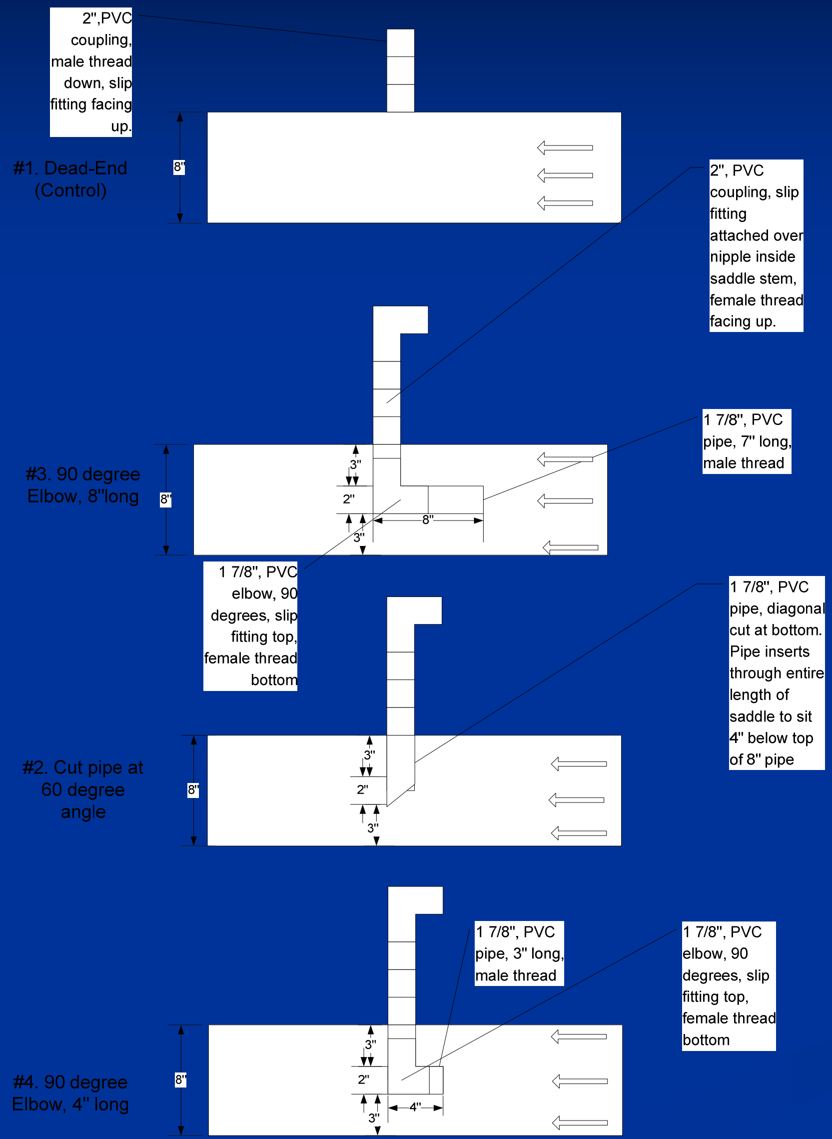
- Laser Obfuscation Particle Counter
- Calibrated by Manufacturer and On-site with latex beads.
- ISO 12103-1, A3 MEDIUM TEST DUST
- ISO 12103-1, A4 COARSE TEST DUST



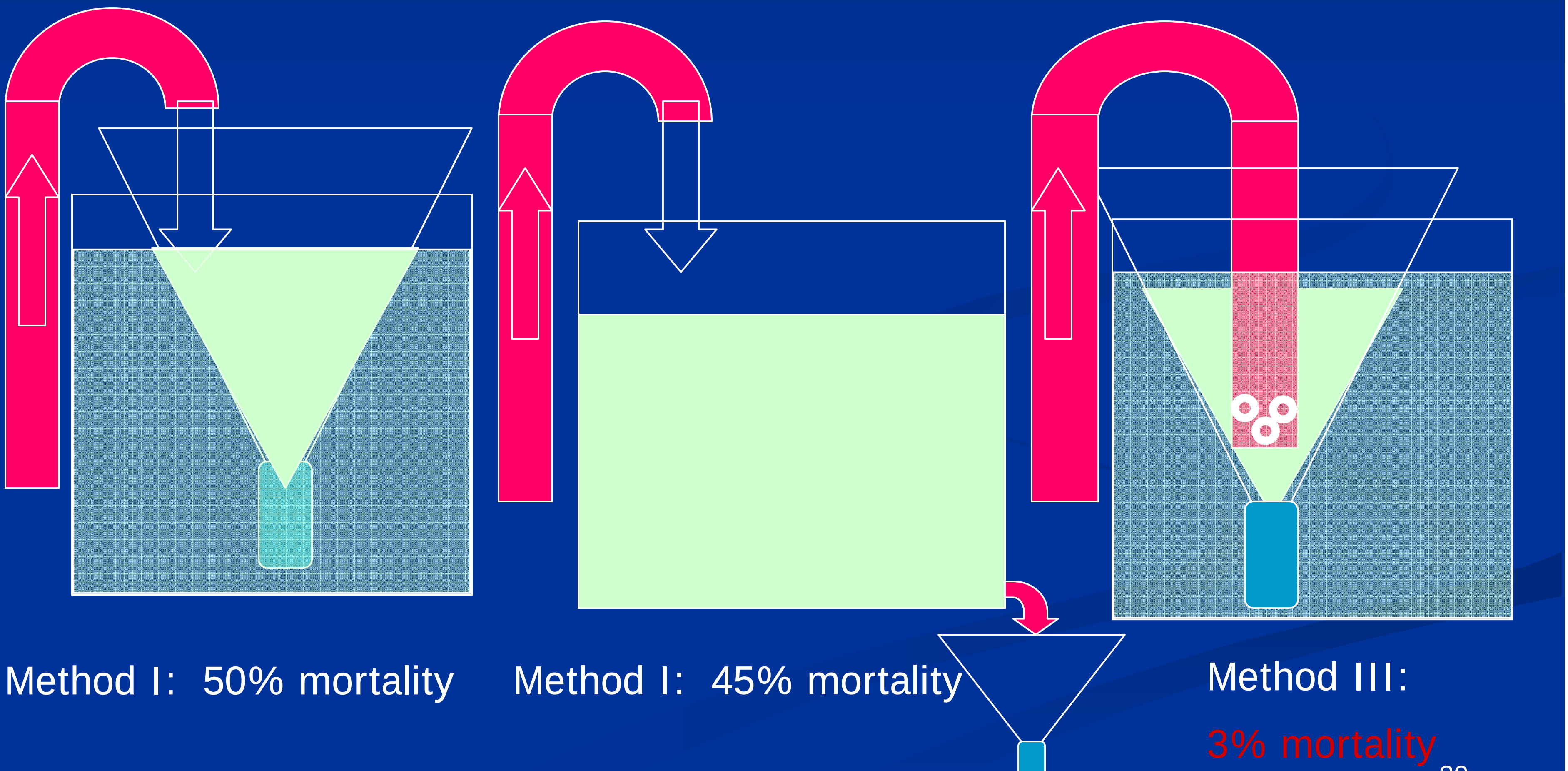


Test Organism Injection

Pipeline Sampling



Sample Collection:



Method I: 50% mortality

Method I: 45% mortality

Method III:

3% mortality

Standard (Surrogate) Test Organisms

- Parallel ETV effort
- Suite of candidates identified by Tech Panel
  - Bacteria
  - Autotrophic protists (single-cell algae)
  - Heterotrophic protists (amoebe w/spores, flagellates)
  - Multicellular plankton (larvae, adults, resistant stages)
- "Cook-off" to identify subset that might best challenge BWT systems (in-progress)
- Other issues also important in determining utility as surrogates
  - Availability, cost, detection/viability, tolerance of test facility, etc.

The ETV Surrogate-species Team

Don Anderson (phytoplankton ecologist, Woods Hole Oceanographic Institution)

Jeffery Cordell and Adelaide Rhodes (zooplankton ecologists, University of Washington)

Fred Dobbs (project manager, Old Dominion University)

Russell Herwig (bacteriologist, University of Washington)

Andrew Rogerson (protozoologist, NOVA Southeastern University)

Surrogate Species List

Functional Group	Fresh Water	Marine Water
Bacteria	Same as marine water.	*Geobacillus stearothermophilus* *Clostridium perfringens* *Enterococcus avium* *Vibrio cholerae*
Heterotrophic Protists	*Acanthamoeba* sp. *Tetrahymena pyriformis* *Vanella anglica* *Vannella platypodia*	*Acanthamoeba* sp. *Paraphysomonas imperforata* *Paraphysomonas vestita* *Uronema marinum*
Phytoplankton	*Fragilaria crotensis* *Peridinium cinctum* *Prymnesium parvum* *Microcystis aeruginosa*	*Chlorella* sp. *Chaetoceros* sp. *Skeletonema costatum* *Scrippsiella lachrymosa* *Scrippsiella trochoidea* *Fragilaria pinnata*
Zooplankton	*Daphnia pulex* *Daphnia magna* *Brachionus calyciflorus* *Moina* sp. *Sinocalanus doerri* *Culex* (insect larvae)	*Eurytemora affinis* *Tisbe* cf. *furcata* *Artemia salina* Mussel and/or oyster larvae

Treatment Stressors and Experimental Conditions

Treatment stressor	Vendor or source	Concentration or Intensity	Exposure time	References
Thermal treatment	Water bath	35º, 40º, 45º, 50º C	4 h	Hallegraeff et al. 1997; Rigby et al. 1999
Chlorine (hypochlorite)	Chlorox bleach	Aqueous solution of sodium hypochlorite. Final concs. of 0.25, 0.5, 1.0, 2.0 mg/L	24 h	Sano et al. 2004; Bolch and Hallegraeff 1993
Chlorine dioxide	Ecochlor, Inc.	Final concs. of 1, 2, 4, 6 ppm	24 h	T. Perlich, Echochlor, Inc. (pers. comm., 19 & 20 Oct. 2004)
Glutaraldehyde	Fisher Scientific	Final concs. of 50, 100, 500 and 1000 mg/L	24 h	Sano et al. 2003
UV light	UV collimator designed and built by Dr. E. "Chip" Blatchley, Purdue University	UV light (256 nm) at 10, 25, 50, 100 mJ/cm^2	Dose is independent of exposure time between approx. 30 sec. to 2 min.	Azanza et al. 2001; Montani et al. 1995; Sutherland et al. 2001; Sutherland et al. 2003
Ozone	Aquatic Eco- Systems, UV-type, 2.4 g with air. Connected to tubing and an air stone.	Total initial residual oxidant (TRO) level of 0.25, 0.5, 1.0, and 2.0 mg Br_2/L.	24 h after achieving initial level of TRO	Hoigné 1998; Langlais et al. 1991; Cooper et al. 2002
Hydrogen peroxide	Fisher Scientific	Final concs. of 0.5, 1, 10 and 20 ppm	24 h after achieving intial concentration	Kuzirian et al. 2001
Deoxygenation	Sparge with N_2 (95%) and CO_2 (5%) mixture, at levels to reduce pH to 5.5, then seal container.	Anoxia (0 mg/L oxygen)	12, 24 , 48, 72 h	Tamburri et al. 2002; P. D. McNulty, NEI Treatment Systems, Inc. (pers. comm., 27 Oct. 2004)
SeaKleen®	Vitamar, Inc.	0.25, 0.5, 1.0, 2.0 mg/L active ingredient	24 h	Cutler et al. 2003; Sano et al. 2004
PeraClean Ocean™	Degussa AG	Final concs. of 50, 100, 200 and 400 ppm	24 h	http://dmses.dot.gov/docimages/pdf81/175321_web.pdf

Analysis

The response variable: percent mortality relative to controls.

Compare % mortality among species/stressor combinations: identify redundancies.

Reduce number of species

Nota Bene

- Experiments are intended to guide selection of the most appropriate organism(s) for testing.
- The selected species will not necessarily be the most resistant species or life stage, since other factors must be considered:

  commercial availability

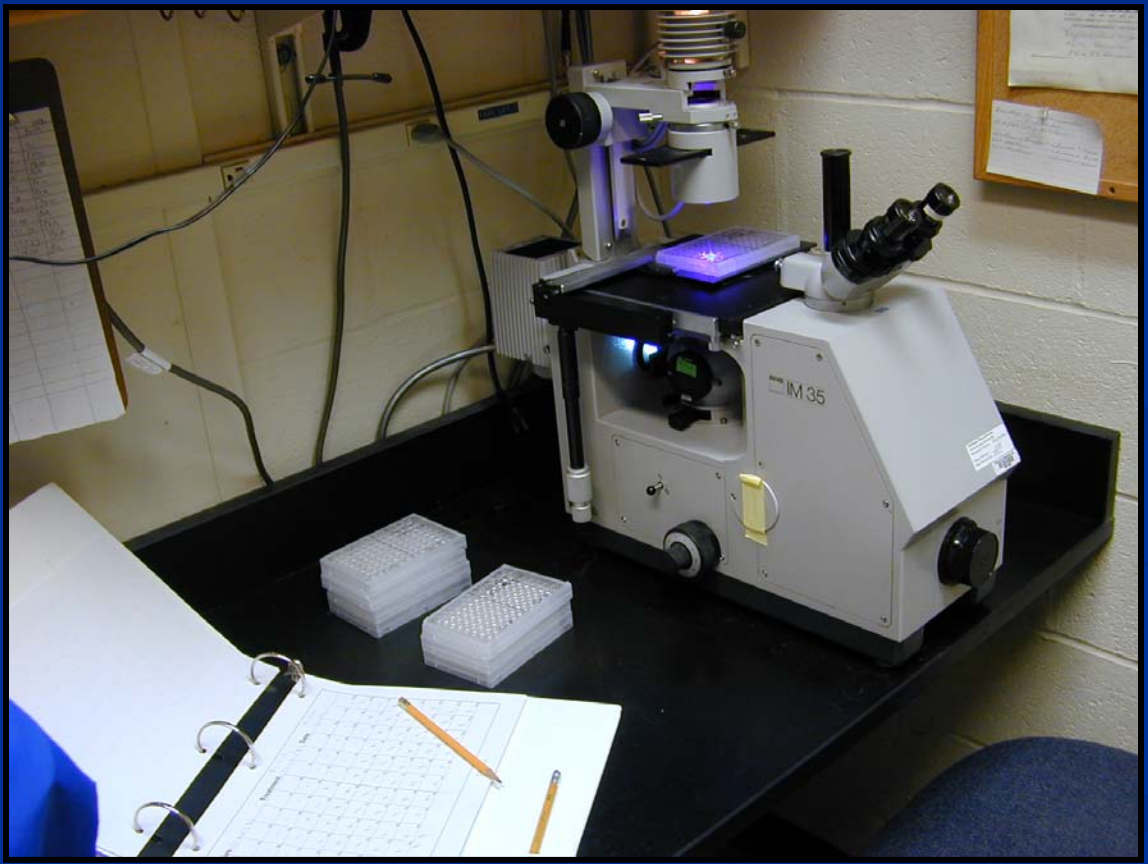
  ease of growth and viability assessment

  susceptibility/resistance) to stressors
- Several surrogates will likely be needed

  provide a composite assessment

Critical need for new analytical methods

The expert at the 'scope is neither sustainable nor desirable



Standardization

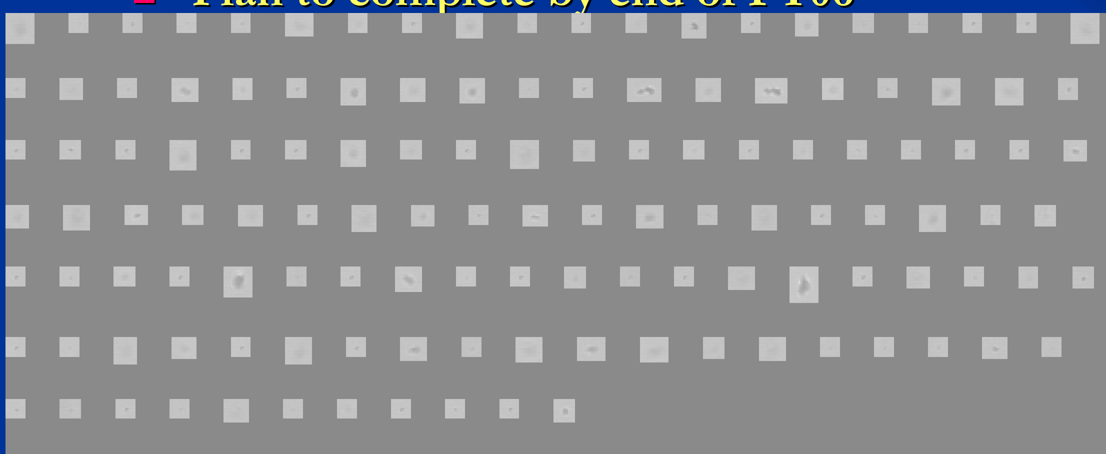
Fatigue

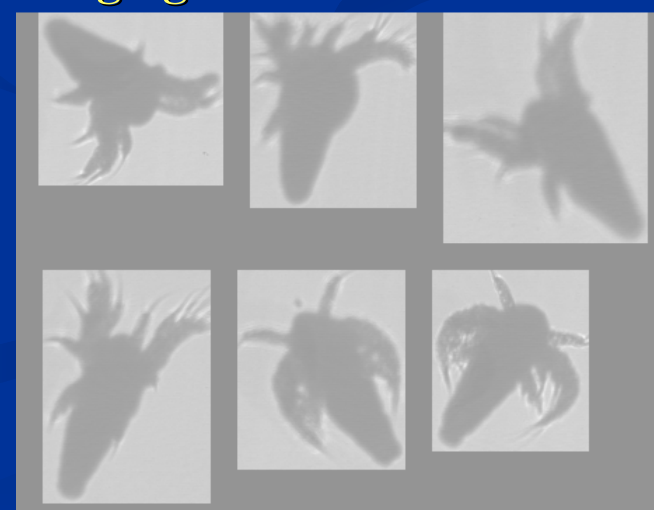
Cost

Time

Automation of Sample Analysis

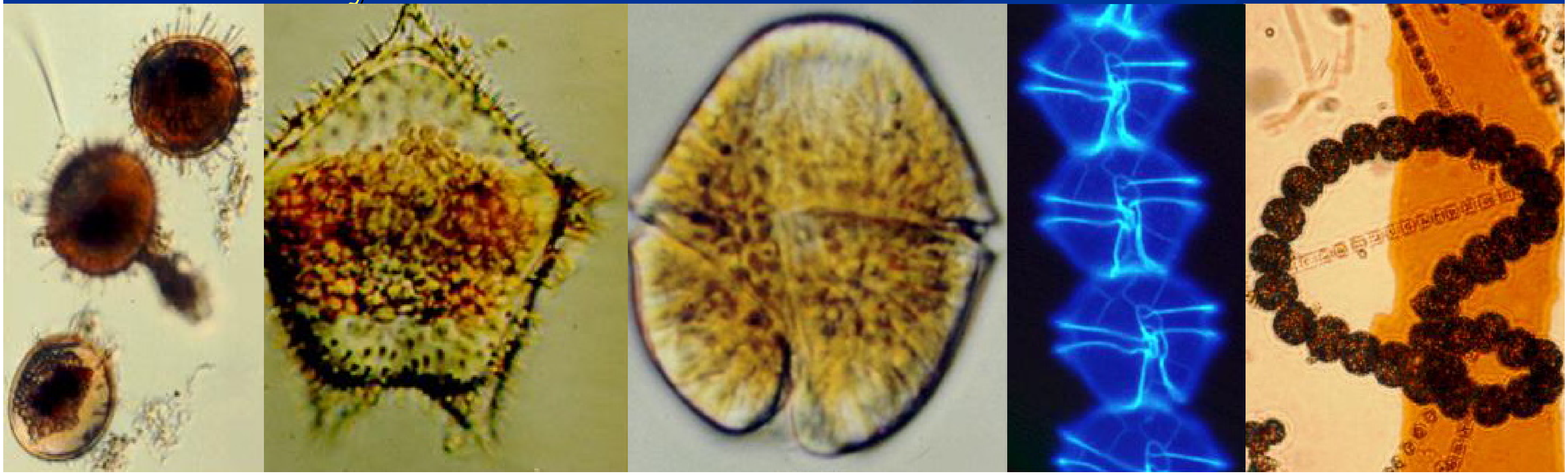
- FlowCAM by Fluid Imaging Technologies Inc.
  - 1 um to 3 mm optical flow cytometer
- In-house Image Analysis
  - Larger plankton
- For both:
  - Incorporation of image categorization
  - Incorporation of dyes for live/dead analysis
  - Possible incorporation of 2nd camera for 2 x 2D imaging
  - Plan to complete by end of FY06





Dinoflagellate Cyst Project

- Attempt to "scale up" cyst production
- Determine the "maturation interval"
- Develop a method for long-term storage
- Develop a rapid and accurate protocol for viability



Attempt to "scale up" cyst production of the most promising surrogate species

- 635,000 liters (700 tons) in ETV facility
- Target concentration of 10 cysts/liter of challenge water
  - Assume a concentration step (~1000x) prior to viability testing

==>6.35 x 10^6 total cysts needed for a test

At current concentration capability we need at least 63.5 liters of culture. (Hard)

If we can produce 1000 cysts per ml of culture, this would only require 6.35 liters of culture. (Easy)

Develop a rapid and accurate protocol for assessing cyst viability

Presently, individual cysts are isolated and monitored for germination.

<u>Problems:</u>

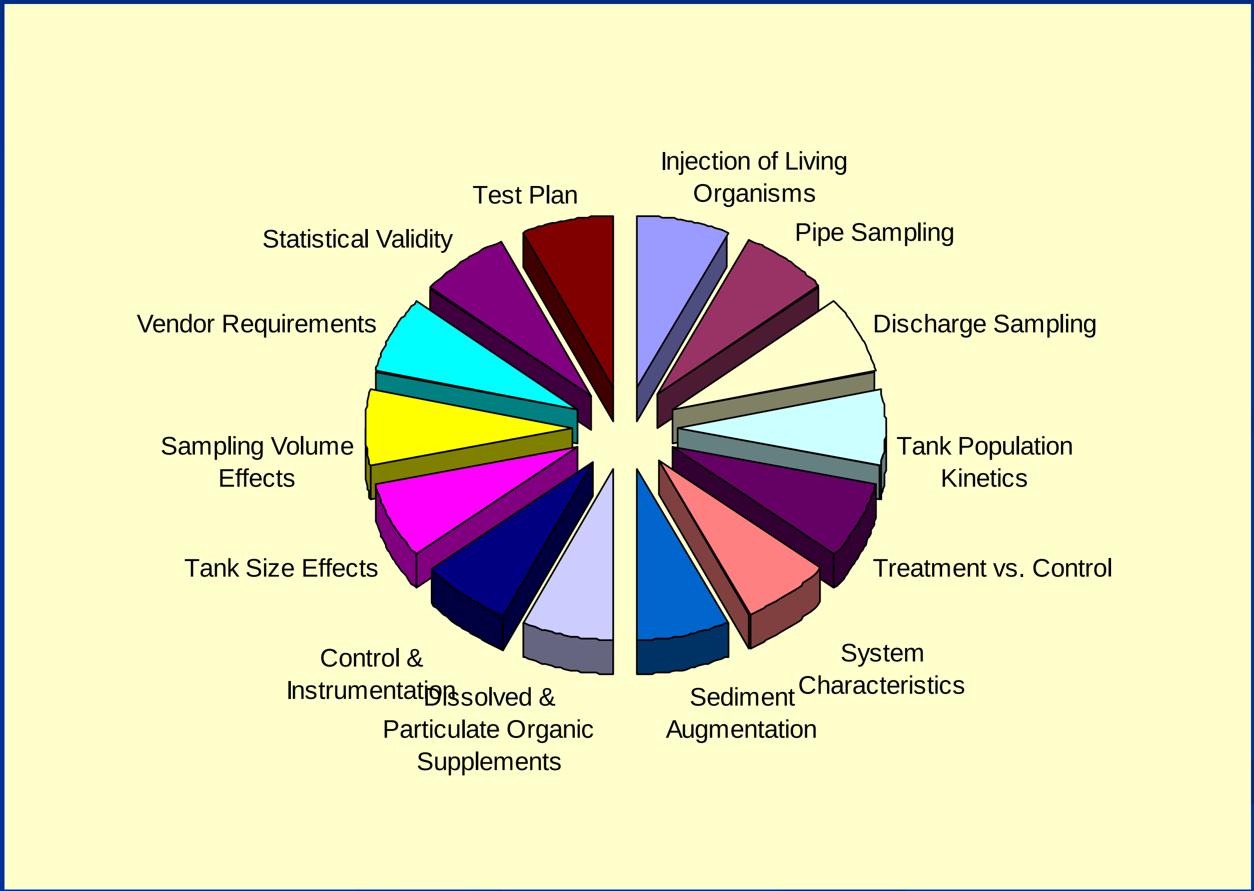
- Very tedious and time consuming
- Variable germination rates for cysts isolated from the same lot on different dates
- Low % of easily observable progeny

==> Assess use of viability probes

- Flow cytometry
- Fluorescence microscopy

"Solving for the Pieces of the Pie"*

- Validation of BWT testing methods has not been done anywhere in the world at full-scale, in a standardized format and with statistical rigor.
- There are fundamental questions and challenges regarding "HOW-TO" perform the testing



* Ted (can I mix you up a metaphor?) Lemieux

http://www.uscg.mil/hq/g-m/mso/estandards.htm

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Things to Consider

- The test results must be
  - Rigorous
  - perhaps legally defensible
- Analytical biological tools are paramount for economically feasible evaluations of treatment technologies.
  - Production time scales
  - Large quantities
  - High statistical confidence and rigor
  - High quality assurance
- Surrogate identification & optimization work required.
- Mirror or comparable test sites (considerable effort) are desirable and likely necessary for reliable and consistent testing in the future.

Things to Consider

- WHO WILL BE QUALIFIED TO PERFORM THESE TESTS?
- WHAT TESTING STANDARDS WILL THEY OPERATE TO?
- HOW DO WE INSURE CONSISTENCY & RELIABILITY AMONGST TEST FACILITIES, DOMESTICALLY & INTERNATIONALLY?