NRL Center for Corrosion Science and Engineering

Validation of Biological Methods for Full-Scale Treatment Testing

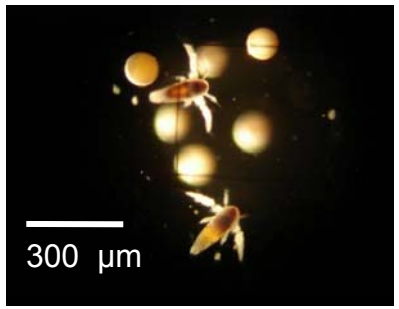
Scott C. Riley, Edward J. Lemieux, Jon F. Grant, Stephanie H. Robbins, Wayne B. Hyland, and Luke E. Davis

16th International Conference of Aquatic Invasive Species
Montreal, Quebec, Canada, 2009

Ballast Water Treatment Test Facility (BWTTF)

Objective of work

- To develop and refine methods for testing the efficacy of ballast water management systems.
  - To collect in-line samples with minimum damage to organisms.
  - Avoid interaction between treatment process effects and biological analyses.
  - Increase the precision of counting organisms.



- Specifically:

I. Quantify the effects of pneumatic diaphragm pumps on the mortality of the test organism *Artemia franciscana* during drain operations.

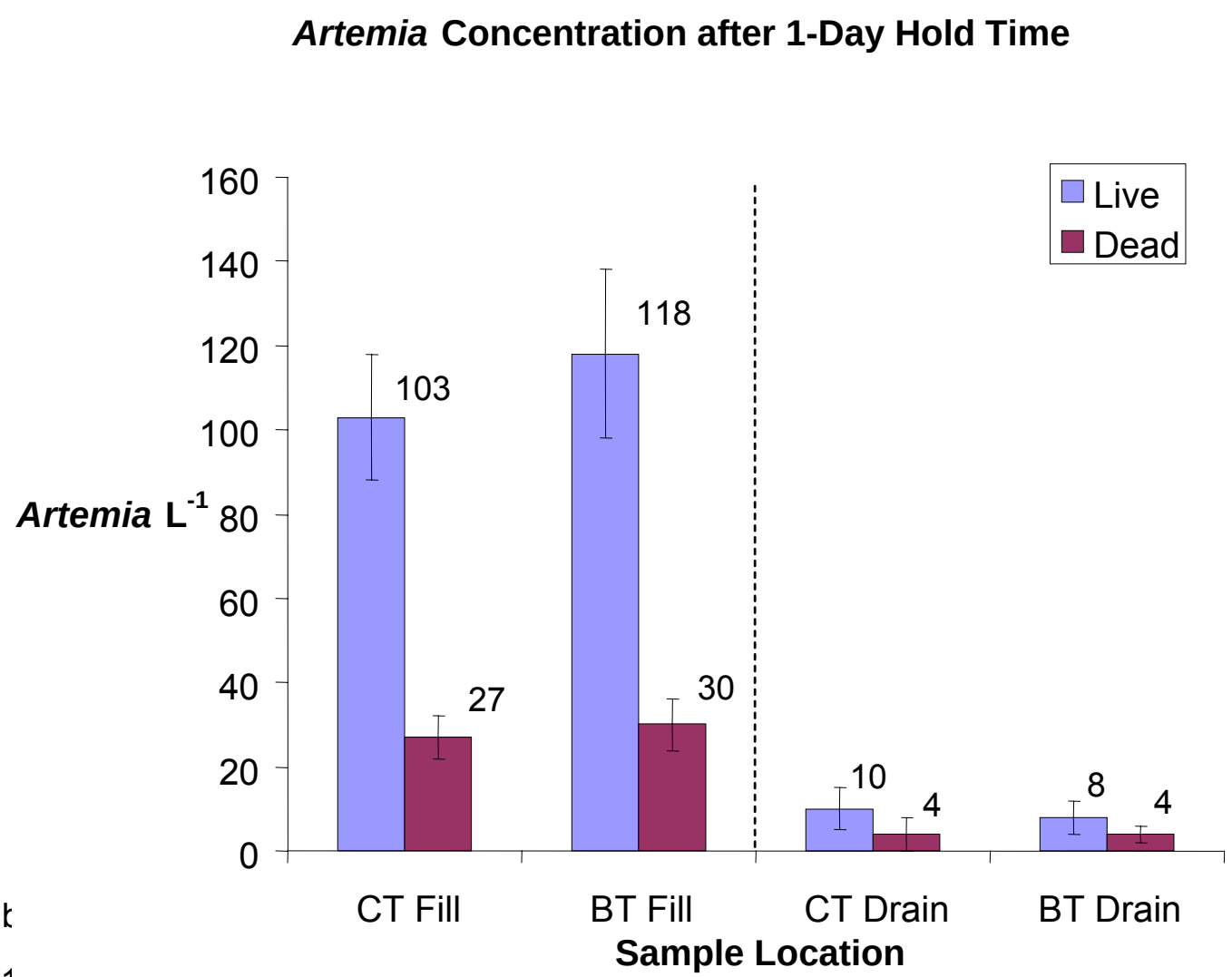
II. Examine the effects of the treatment agent chlorine on phytoplankton cell labeling by the fluorescent , mortal stain SYTOX Green®.

III. Compare de-mobilization agents of motile phytoplankton (de-mobilization is necessary to generate representative measurements).

I. BWTTF Drain Operations by Gravity

- Initially, during drain operations, the head pressure created by gravity was the motive force for draining each tank.
- As the CT/BT tank levels dropped, the in-line sample flow rate slowed down to the point of reversing, creating a siphon causing sample flow out of the collection tanks.
- This phenomenon resulted in sample loss and prevented the collection of time-integrated drain sampling over the entire tank volume.

I. BWTTF Drain Operations by Gravity

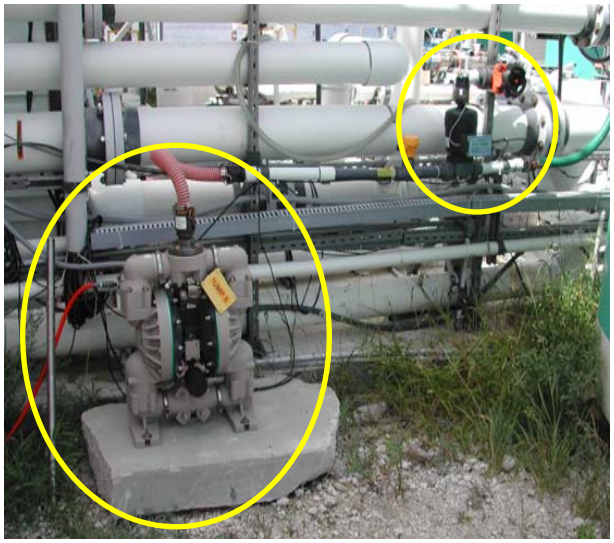


N= 3 Sub

Bars = 1 SD

ICAIS, 2009

I. Pneumatic Diaphragm Pump vs. Centrifugal Pump



Pneumatic Diaphragm Pump

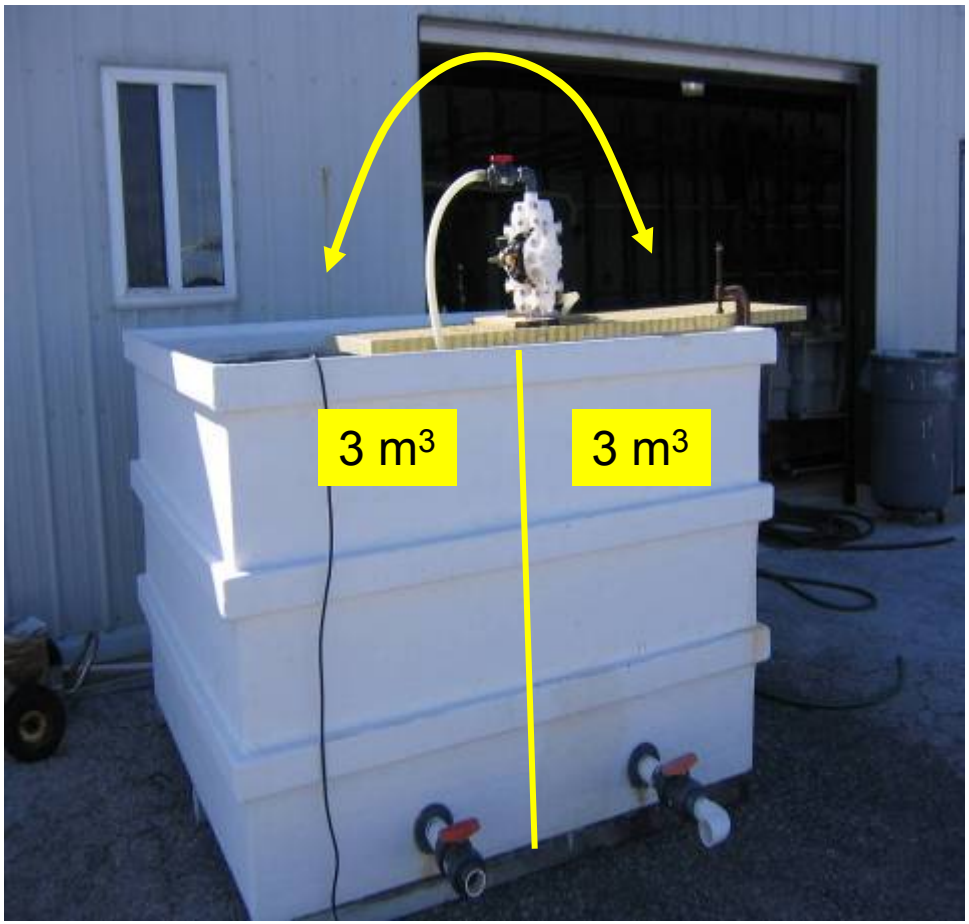
- Low shear pump.
- Air diaphragms insure positive flow.
- No sharp edges; decreasing possible damage to organisms.
- Diaphragms are made of Teflon, limiting the possibility of organisms getting stuck.

Centrifugal Pump

- Centrifugal pump has an impeller.
- Violent spinning motion by impeller can cause organism damage.

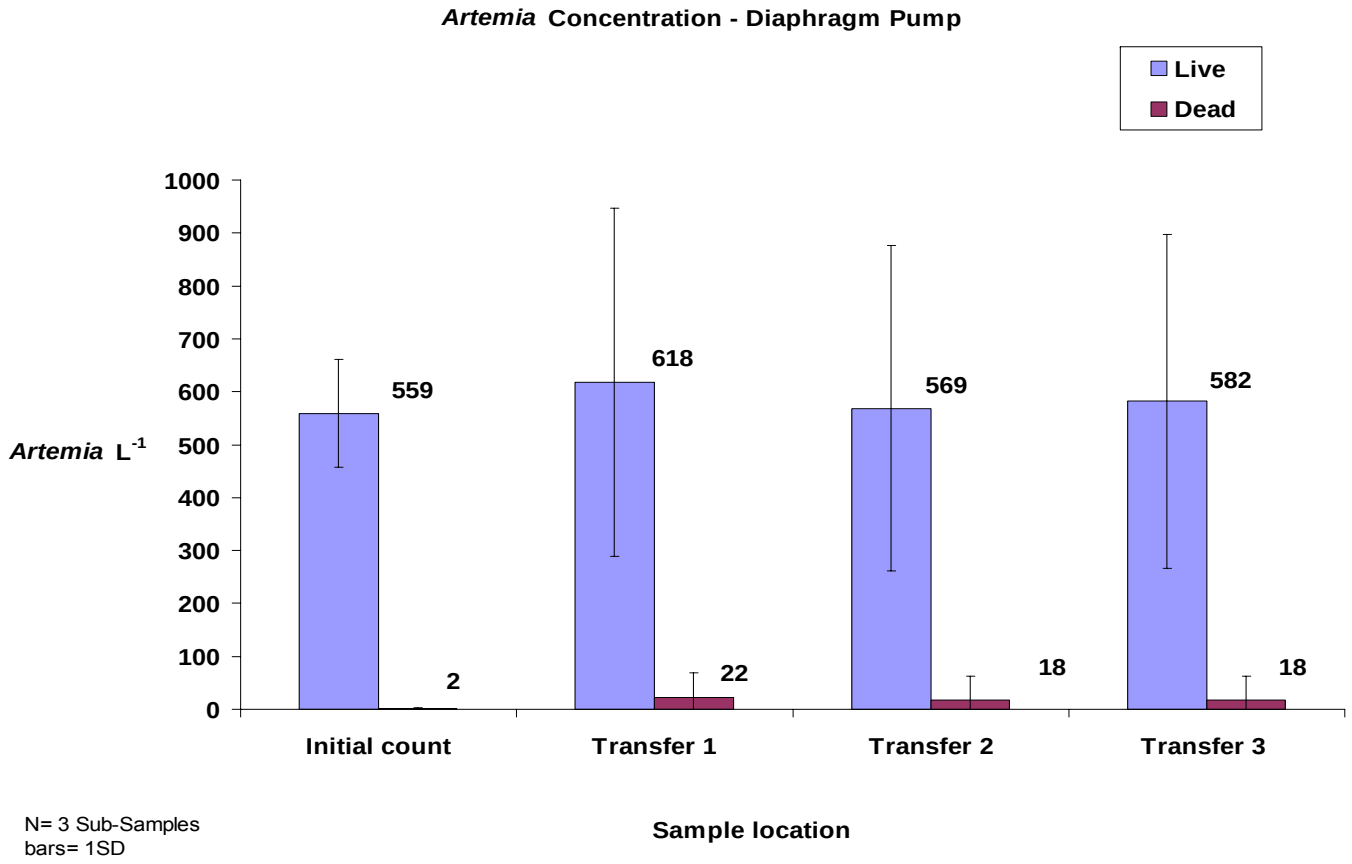
I. Mortality Effects of Pneumatic Diaphragm Pumps

- Two 3 m^3 sample tanks side by side, with a diaphragm pump set in-between transferring 24 hour old *Artemia* in filtered seawater from one sample tank to the other.
- Using a 1.5 liter Niskin bottle, three sub-samples were taken at different locations (front, middle, back) from the filled 3 m^3 sample tank.
- This sample procedure was conducted following the initial fill and after three water transfers performed by the diaphragm pump.
- N= 3 transfers with only *Artemia* added.



ICAIS, 2009

I. Mortality Effects of Pneumatic Diaphragm Pumps



- Little change in the mean concentration of dead *Artemia* following the initial count to the three transfers.
- The high percentage of live organisms indicates negligible degradation of *Artemia* during the transfer process by the pump.

ICAIS, 2009

II. Effects of Chlorine on SYTOX Green®–Stained Phytoplankton Samples

- Biological stains are critical in enumerating and determining the viability of organisms.
- Some treatment systems have the potential to interfere with stains, e.g., generation of hypochlorite.
- The ETV Pilot Test was conducted with a chlorination treatment process.

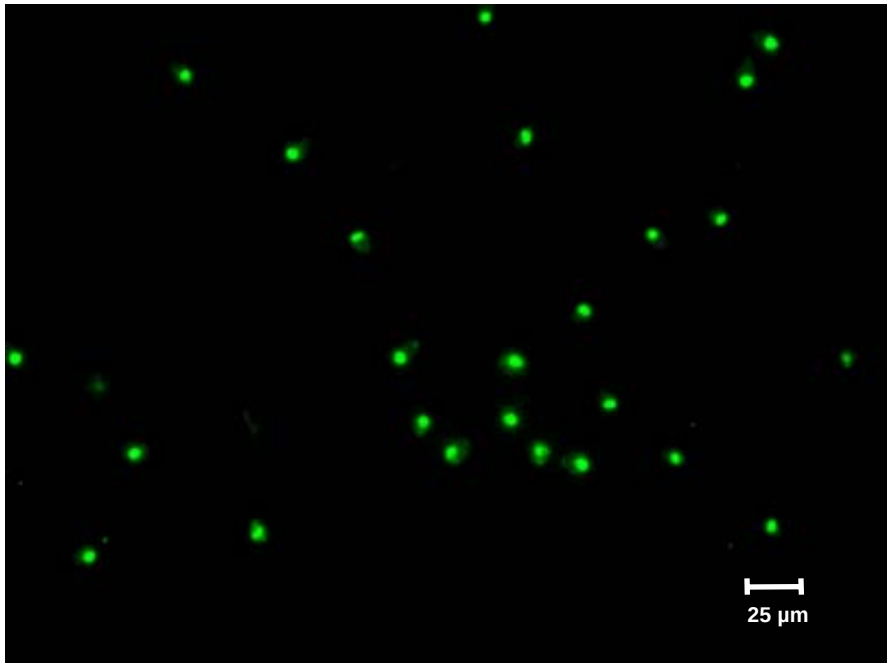
http://severntrentservices.com/denora/balpure/index.aspx

II. Effects of Chlorine on SYTOX Green®–Stained Phytoplankton Samples

- To ensure that residual chlorine would not interfere with the mortal stain SYTOX Green®, the effect of chlorine on stained phytoplankton samples was examined.
  - 5 mg of chlorine agent was added to 200 ml of filtered seawater containing a mix of live and dead test organisms (*Tetraselmis*).
  - The average free chlorine reading of the working stock was 3.8 ppm, typical level for residual chlorine in the ballast tank one day after treatment.

www.livephytoplankton.com

II.SYTOX Green®– Stained Phytoplankton Tetraselmis cells

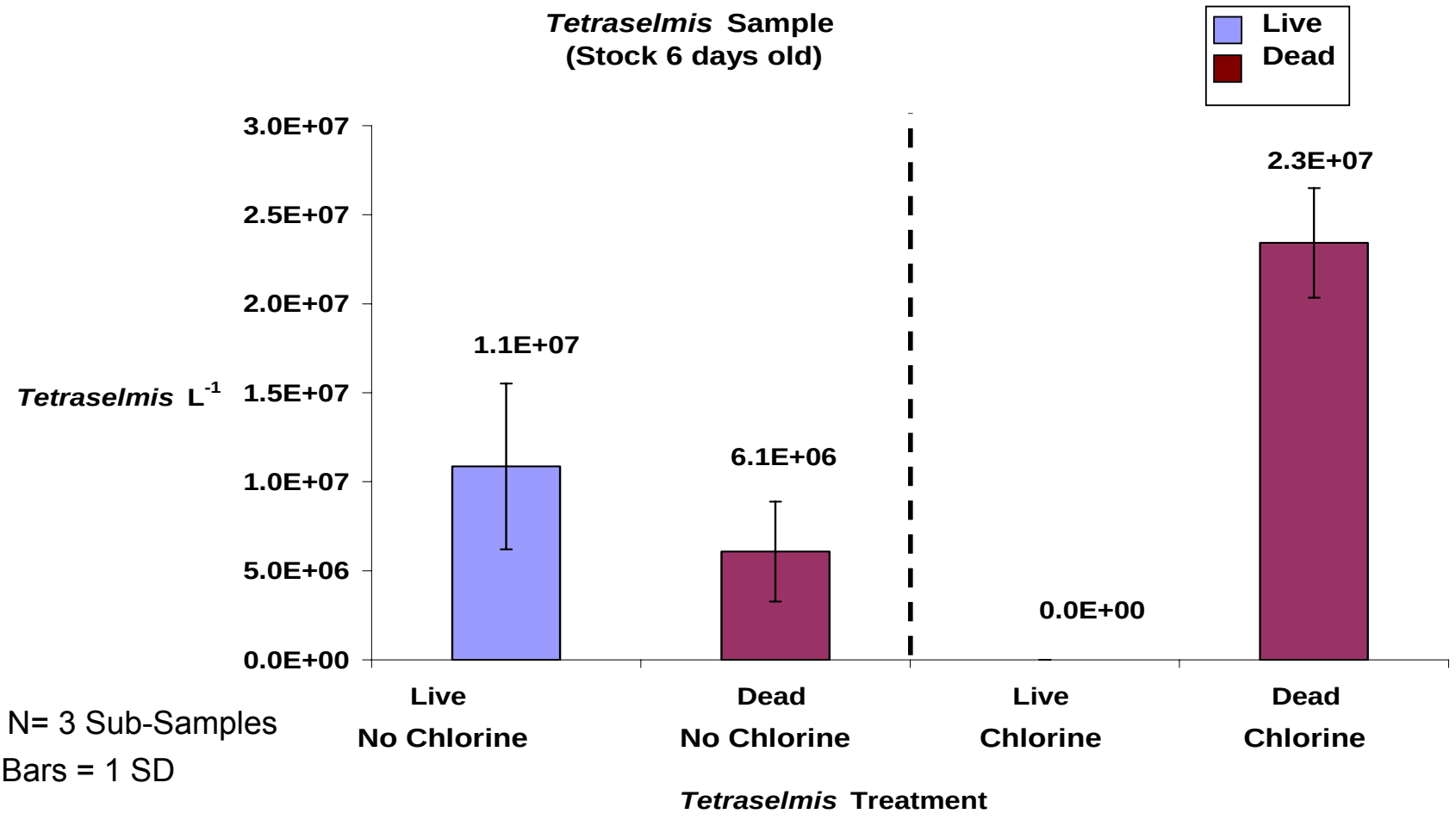


***Tetraselmis* – FITC filter (200X magnification)**

Sedgewick Rafter Slide

- Both control and chlorinated samples were analyzed for live/dead *Tetraselmis* populations using the Sedgewick Rafter method with SYTOX Green®.

II. Effects of Chlorine on SYTOX Green®–Stained Tetraselmis



N= 3 Sub-Samples

Bars = 1 SD

- **Complete mortality of *Tetraselmis* in the chlorine treated sample.**
- **Dead *Tetraselmis* cells were successfully stained with SYTOX Green®.**
- **If chlorine interfered with SYTOX Green®, we would expect lower dead counts in the chlorine-treated sample.**

III. De-mobilization agents

- Large variance was observed in phytoplankton counts during previous studies.
- One possible source of the variance was the inaccurate counting because cells were not adequately de-mobilized.
- The methods for phytoplankton analysis were re-examined.

- Carbon dioxide and acetic acid were two potential chemical constituents to be used to de-mobilize the organisms. Widely available club soda and vinegar were used.
- Fresh cultures of *Tetraselmis* were supplied to NRLKW over a two-week period.

- Club Soda (CS)

• 10 ml *Tetraselmis* + 5 ml of CS → 1 ml for SR.

- Low Acidity Vinegar

• 10 ml *Tetraselmis* + 5 ml of vinegar → 1 ml for SR.

• 10 ml *Tetraselmis* + 0.1 ml of vinegar → 1 ml for SR.

III. De-mobilization agents

Mean *Tetraselmis* counts x10^8 l^{-1}

Sedgewick Rafter (with CS)	Sedgewick Rafter (5 ml vinegar)	Sedgewick Rafter (0.1 ml vinegar)
6.0	9.0*	7.0
7.8	8.6	9.2*
5.6	8.5*	7.6*
2.9	4.7	4.6
6.0	10.0*	8.9*

- The mean counts using CS were significantly different from at least one of the vinegar treatments in 4 of 5 trials ($p < 0.05$; two-way ANOVA on rank-transformed data; differences are marked by *).
- The data indicate that vinegar is the better of the two agents to de-mobilize motile phytoplankton.

Conclusions

- The Mortality Effects of Pneumatic Diaphragm Pumps
  - Pumps are necessary to prevent siphoning and maintain flow.
  - The diaphragm pump exhibited no damage to *Artemia*.
- Effects of Chlorine on SYTOX Green® – Stained Phytoplankton Samples
  - The addition of chlorine resulted in complete mortality of *Tetraselmis* cells.
  - The chlorine used to treat the 6-day old *Tetraselmis* stock had no discernable effect on the functioning of the mortal stain.
- De-mobilization agents
  - Results indicate that vinegar is the better of the two de-mobilization agents.
- Site-specific validation is recommended.

Acknowledgements

U.S. Coast Guard Environmental Standards Division (CG-5224)