Title
AQU2: Detection and Identification of Aquatic Microorganisms

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Detection and Identification of Aquatic Microorganisms

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Introduction: Understanding and observation of aquatic microbial populations

Ecologically important marine microorganisms

- Harmful Algal Blooms
  - Blooms that are toxic to marine life and harmful to human health are increasing nationally and globally.
  - Many bloom-forming algae are small in size and patchy in distribution, making detection and identification problematic.
  - The conditions under which blooms occur and subside are still poorly understood and require massive sampling efforts on both spatial and temporal scales.

Model systems

- Aureococcus anophagefferens
  - A. anophagefferens is a small (2-3 µm) spherical cell which is the cause of so-called ‘Brown Tides,’ discolorations of the waters off the Mid-Atlantic coast.
  - Brown Tides have serious impacts on shellfisheries and water quality.

- Lingulodinium polyedrum
  - L. polyedrum is a bloom-forming dinoflagellate that causes so-called ‘Red Tides’ off the California coast.
  - Blooms of L. polyedrum have been associated with fish and shellfish mortality events

Problem Description: Understanding aquatic microbial population development

Experimental studies in laboratory testbed

- Artificial stimulation of a ‘brown tide’ in a thermally stratified column with a demonstration of predation effects
- Monitoring of diel vertical migration of a red tide dinoflagellate, L. polyedrum, in a thermally stratified column

Microorganism sensing and identification

- Flow cytometric identification & enumeration using a fluorescently-labeled monoclonal antibody specific against A. anophagefferens
- Identification of single A. anophagefferens cells using atomic force microscopy (AFM) and antibody-functionalized tips
- Fabrication of nanowire and carbon nanotube sensors and demonstration of sensing principles for A. anophagefferens

Proposed Solution: Laboratory-based population studies and unique detection techniques

Approaches

- Artificial stimulation of Brown Tide in column testbed
  - A thermally stratified column was inoculated with a culture of A. anophagefferens and its growth was monitored over several weeks.
  - After maximum growth was attained, a predator, Pedinella sp., was added to the column and the population dynamics were followed using flow cytometry and microscopical techniques.

- Study of diel vertical migration of Red Tide dinoflagellate in column testbed
  - A thermally stratified column was inoculated with a culture of L. polyedrum and its relative vertical position was studied over the course of a week.
  - L. polyedrum showed distinct vertical migration over a 24-hour period, accumulating in the surface waters early in the morning and dispersing throughout the column at night (Figure 1).

- This experiment also allowed for direct comparison between a newly-developed QPCR technique and more classical microscopical techniques for enumeration of L. polyedrum cells.

- Immuno-based flow cytometric approach for enumeration of A. anophagefferens
  - A fluorescently-labeled antibody specific to A. anophagefferens was used to detect cells in natural seawater samples.

- This technique is now used for routine analysis of natural water samples (Figure 2A).

- Detection of A. anophagefferens using AFM and antibody-functionalized tips
  - A. anophagefferens cells were immobilized on an Si/SiO2 surface using PEI and the specific monoclonal antibody; AFM tips were functionalized with antibody using an ethanolamine approach.
  - Over 100 force-distance measurements were made of single cells on surfaces containing immobilized cells and surfaces with blocked, non-reactive cells. The f-d curves show a definite difference between these surfaces (Figure 2B).

- Nanowire and carbon nanotube sensing of A. anophagefferens
  - Successfully synthesized single-walled carbon nanotubes and a variety of novel nanowires based on In2O3, SnO2 and CdO.
  - Preliminary tests to confirm the capability of our nanotube transistors to detect A. anophagefferens, showing a drop in conductivity following the addition of cells (Figure 3)