

# Methods for Quantifying Biofouling: An Initial Examination of Optical and Acoustic Approaches

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ICAIS • 22-26 OCT 2017 • Ft. Lauderdale

# **Biofouling Background**

- Potential transport of aquatic nuisance species
- Major concern for vessels with operating profiles with long periods of inactivity\*
- Currently, international guidelines have been promulgated for the reduction and prevention of biofouling





https://www.green4sea.com/amsa-revises-biofouling-and-in-water-cleaning-guidelines



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### **Fouling Mitigation—Coatings**

### Before 1960

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 Toxic biocides (for example arsenic, mercury, and DDT) used in coatings to kill attached organisms

### After 1960

- Tributyltin (TBT)
  - Toxic and persistent in environment
  - Worldwide ban on application since 2003
    - International Convention on the Control of Harmful Antifouling Systems on Ships (International Maritime Organization, 2001)
    - Currently, coatings with other antifoulants are used (e.g., copper)
    - Other approaches being used (e.g., fouling-release coatings)



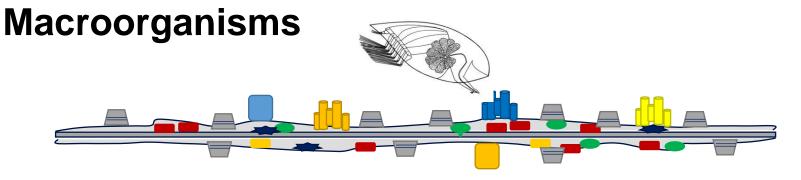
U.S. NAVAL RESEARCH LABORATORY Biofouling Organisms

#### Microorganisms

**Biofilm formation:** 

Surface immersed in seawater

- Microorganisms (e.g., bacteria and microalgae) adhere to submerged surfaces and produce extracellular polymeric substances (EPS)
- Other organisms (protists, fungi, microinvertebrates) accumulate in the biofilm
- The thickness is on the scale of micrometers to millimeters



- Examples of hard foulers: Larval barnacles settle as cyprids undergoes metamorphosis to adult barnacle, tube worms
- Examples of soft foulers: Macroalgae, tunicates, sponges



### **Goal of Work**

- The purpose of this pilot study was to evaluate optical and acoustic approaches to quantifying biofouling
- Methods Tested:
  - Imaging Fluorometry
  - Acoustic Imaging (with single and dual beam sonars)
  - Optical Imaging (using an underwater, digital camera)

# Note: Methods not primarily designed for biofouling quantification

### **Methods**—Test Panels and **Exposure Sites**

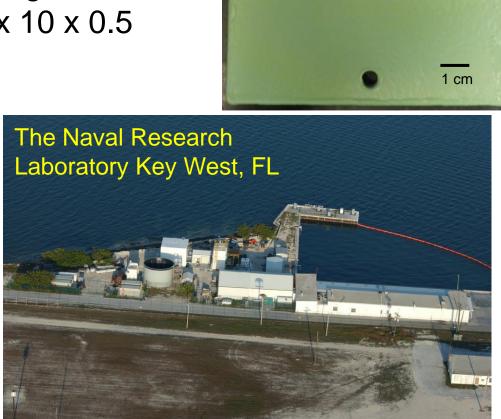
#### Test panels provide substrate for PSM

biofouling growth

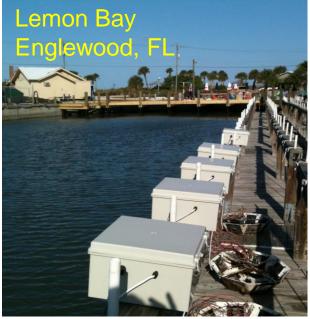
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ABORATORY

Steel test panels with inert epoxy coating (10 x 10 x 0.5 cm)







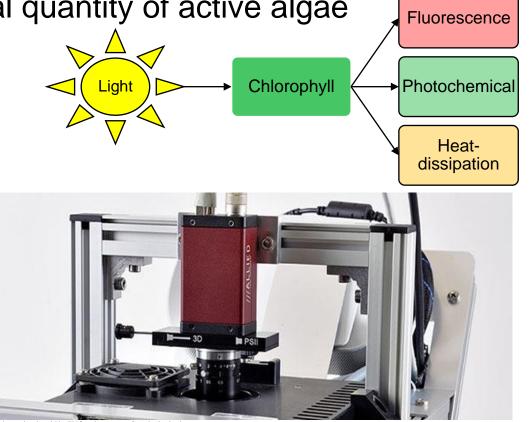
## **Methods—Imaging Fluorometry**

- Variable fluorescence fluorometry
  - Saturation Pulse Method

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- Basic assessment of photosynthetic performance of a sample
  - Photochemical yield  $(F_V/F_M)$
- Can provide information on total quantity of active algae
- Imaging-PAM (MAXI version; WALZ, Germany)
  - Maps fluorescence signals along surface using digital camera
  - Collects fluorescence characteristics defined by pixels
  - Generates high-resolution
    2D map (algae biofilms)



http://www.walz.com/products/chl\_p700/imaging-pam\_ms/introduction.htm

#### U.S. NAVAL RESEARCH Methods—Acoustic Imaging

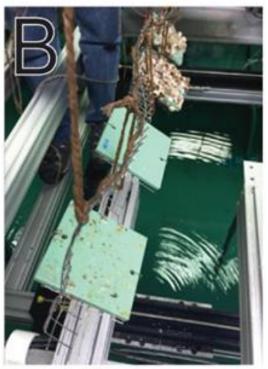
- 2D Imaging Sonar Single Beam
  - M450 2D Imaging Sonar; BlueView; Bothell, WA
  - Single frequency 450 kHz

Single beam sonar approach:

A. Test panels held in wire mesh

B. Lowered into tank

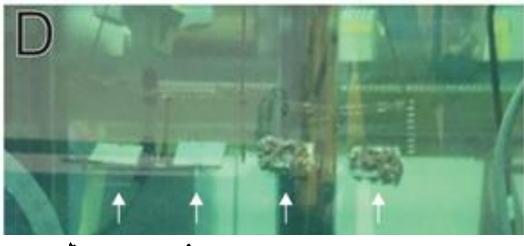




#### U.S. NAVAL RESEARCH LABORATORY Methods—Acoustic Imaging -Continued

### Single beam sonar approach:

- C. Suspended panels (view through tank)
- D. Outlined region is enlarged to show the panels





Heavy Heavy

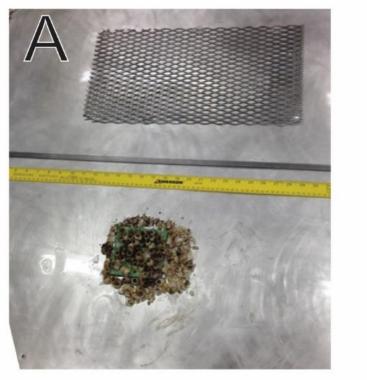


### Methods—Acoustic Imaging -Continued

- 2D Imaging Sonar Dual Beam
  - M900-2250 Imaging Sonar; BlueView, Bothell, WA
  - Mid frequency 900 kHz; high frequency 2250 kHZ
    Dual beam sonar approach:
- A. Wire mesh used as reference

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B. Barnacles scraped from one side allowing panel to sit flat; Scraped barnacles were piled on and near top of panel





Panel were submerged in the test tank for acoustic imaging with a dual-beam sonar

### **Methods—Optical Imaging**

### Methods

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- Waterproof, digital camera used for underwater imaging
- Panels submerged for short (40 d) and long-term (640 d) exposures
- Periodic imaging in flow-through troughs to capture fouling rates
- Fouled panels compared to a non-fouled control surface

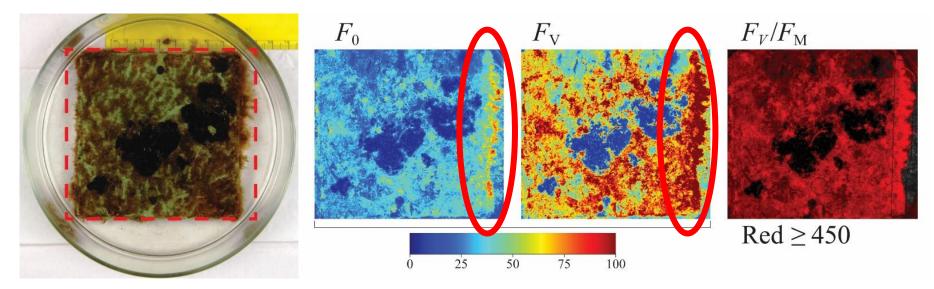
Lumix DMC-TS5 (Panasonic North America, Newark, NJ)





Flow-through troughs used to hold panels during imaging in Key West, FL

### **Results—Imaging Fluorometry**



- Visible distribution of algal films throughout panel
- Dark region in center of panel (colonial tunicate) displayed low fluorescence values
- Filamentous algae on right edge of test panel displayed high relative fluorescence intensities
  - Pixels with  $F_V/F_M > 450$  (no units) were labeled red

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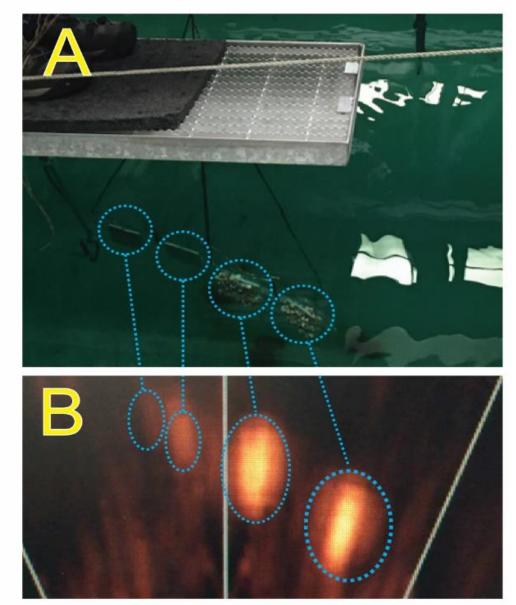
### **Results—Acoustic Imaging**

A – B.) Single-beam acoustic imaging (low frequency) could distinguish among panels based on signal intensity

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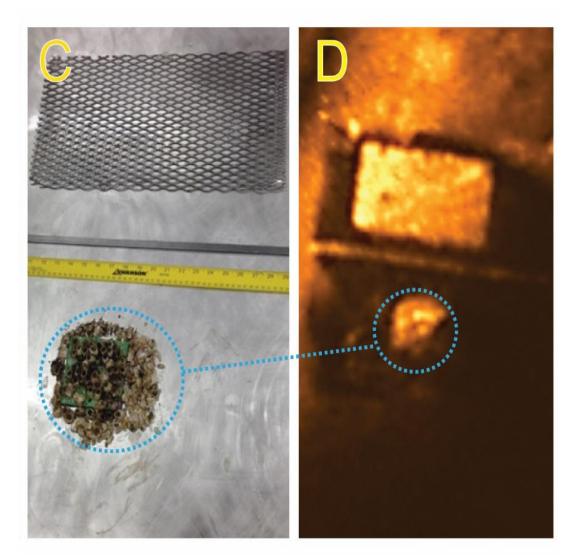
The resolution was not high enough to distinguish difference within a panel



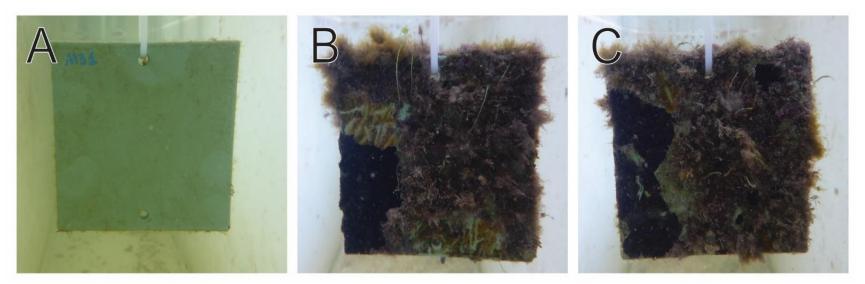
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### **Results—Acoustic Imaging -Continued**

C- D.) The dual-beam acoustic imaging (highfrequency) showed higher resolution, and regions within the panel could be distinguished based upon the signal intensity



### **Results—Optical Imaging**



- High resolution images of both algal biofilms and mixed assemblages were collected (A: 40 d incubation; B-C: 640 d incubation)
- Differences between images reflects the affect of water quality on imaging
- Work is currently underway with colleagues from Old Dominion University (ODU) to develop image processing techniques to categorize general taxa and measure surface roughness based on pixel values

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### Conclusions

- Imaging fluorometry requires test panels to be evaluated ex situ; targets only phototrophic organisms
- Technologies may be suited for different tasks
  - Imaging fluorometry measures photochemical yield; could be used to monitor the efficacy of surface treatments or it could be combined with other approaches used to interrogate surfaces
- Single-beam acoustic imaging could distinguish difference among panels (minimal – heavy fouling), but could not distinguish difference within a panel – not enough resolution

# **Conclusions—Continued**

- Regional differences within a panel could be distinguished based on signal intensity due to the higher resolution of the dual-beam acoustic imaging
- Optical images collected under ideal conditions indicate the degree of fouling and allowed for identification of organisms in a mature community (data in prep.)
- Cameras are more available, cheaper, and easier to operate than acoustic-based systems
  - Still require software development to measure quantity of biofouling

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### **Acknowledgements**

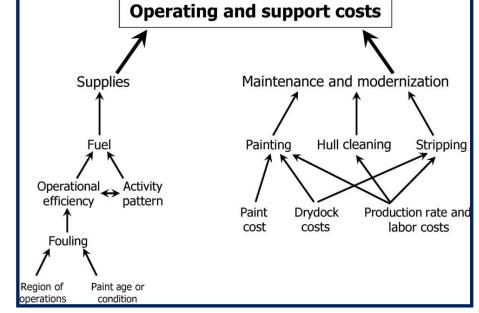
This work was funded by the Environmental Protection Agency, agreement # DW-17-92399701

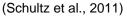
We greatly appreciate the advice and programmatic support of **Robin Danesi** and **Jack Faulk** (EPA)



### Background

- Increased frictional drag
  → increased fuel costs
- Typical fouling levels →
  ~10% increase in fuel
  consumption\*
- Hull maintenance costs (cleaning, applying new coatings)
- Estimated overall costs of hull fouling: \$56M y<sup>-1</sup> for DDG-51 class\*
- \* Schultz et al., 2011







https://en.wikipedia.org/wiki/USS\_Arleigh\_Burke



- Logistics
- No specified interval for hull cleaning